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Altering sweet potato starch functionality by amino acids and pH treatments

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ALTERING SWEET POTATO STARCH FUNCTIONALITY BY AMINO ACIDS AND pH TREATMENTS

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

The sweet potato is a vegetable containing high levels of different vitamins and minerals, needed to protect the body against disease. This study focused on starch found in the Beauregard and Evangeline sweet potatoes and observed the effect of pH and amino acid additives altering the functionality of starch. These modifications of the Beauregard and Evangeline sweet potato starches will also be done to determine if an increase in resistant starch and slowly digestible starch is found.

Beauregard and Evangeline starches had similar gelatinization temperature and the two starches required the same amount of energy to gelatinize. In Freeze-dried Beauregard starch, peak temperature decreased with pH treatments and pH10 decreasing peak temperature to 68.7°C. Histidine at pH10 decreased peak temperature to 69.7°C. In Evangeline freeze-dried starch, histidine significantly decreased peak temperature, especially at pH10 for one hour (73.17°C) compared to native. In Beauregard oven-dried starch, the control significantly lowered peak temperature compared to the native. pH3 and 10 were significant in lowering peak temperature of the starch. Lysine and histidine were significant amino acids in decreasing peak temperature. In Evangeline oven-dried starch, histidine at pH3 and pH10 were significant for decreasing peak temperature compared to the native starch.

Positively charge amino acids along with pH treatments caused significant alterations in pasting properties of both Beauregard and Evangeline sweet potato starches. Oven-dried starch was more responsive to changes in pasting characteristics than freeze-dried sweet potato starch. Evangeline oven-dried sweet potato starch, histidine lowered breakdown and with the addition of pH10 treatment breakdown decreased even further, increasing its stability to shear during cooking.

There was a trend towards increased RS with amino acids added at pH3 or pH10 versus pH3 or pH10 alone, especially for lysine and histidine. Freeze-dried Beauregard starch SDS showed large increases with pH3 for 1 hour and lysine and histidine at pH3 for 1 hour. SDS content increased in oven-dried Beauregard starch the most with arginine at pH3. Freeze-dried Evangeline starch SDS content increased the greatest with histidine at pH3. SDS content increased the greatest with lysine at pH3 for oven-dried Evangeline starch.

CHAPTER 1. INTRODUCTION

Starch containing products are one of the main energy sources in the human diet. Starch is the main dietary source of carbohydrates, which can be found in plants and occurs as granules in the chloroplasts of green leaves and in the amyloplasts of seeds, pulses and tubers (Ellis and others 1998). Starch obtained from different sources can have different characteristics which affect the stability of the starch during processing. The food industry requires a variety of starches able to tolerate a wide range of processing techniques. Starch can be modified in order to alter the properties. It has been found that proteins, lipids and amino acids can alter starch properties (Liang and King 2003). An and King (2009) found that ozonation and amino acids altered pasting properties of rice starch. Lockwood and King (2008) found that charged amino acids increased the cooking stability of orange-fleshed sweet potato starch.

Resistant starch and slowly digestible starch are starch characteristics which promote important and biological health benefits. Resistant starch offers similar health benefits of fiber. Foods with a higher content of slowly digestible starch offer a lower glycemic response, which may provide health benefits for fighting diabetes, cardiovascular disease and obesity.

Sweet potatoes are an excellent source of beta carotene, vitamin C, niacin, riboflavin, thiamin and minerals (Zuraida 2003). The sweet potato is a staple crop for many countries due to its durability to withstand climates changes and ability to grow in a wide variety of soil conditions (Ishiguro and others 2003). Louisiana produces 24% of the nation's sweet potato crop on a collective 23,000 acres, with revenues of $105 million (Lucier et al., 2002). The Beauregard sweet potato is the dominant variety grown in Louisiana. Both the Beauregard and Evangeline sweet potatoes were developed at the LSU AgCenter, the Beauregard sweet potato variety by Larry Ralston in 1987 and the Evangeline sweet potato variety by Dr Don Labonte in 2007.

This research studied Beauregard sweet potato starch and Evangeline sweet potato starch. These starches were compared for different thermal characteristics by DSC, pasting

characteristics by RVA, rheological properties by a rheometer, resistant starch content by the Megazyme method and slowly digestible starch content by the Englyst method. Positivly charged amino acids were added based upon previous sweet potato starch research of Lockwood (2005) on a 6% dry weight basis. pH treatments were used to alter the binding ability of the amino acid.

CHAPTER 2. LITERATURE REVIEW

2.1. Starch

Starch products are one of the key energy sources (carbohydrates) in the human diet. Starch is stored as polysaccharides in plants and starch granules are found in the chloroplast of green leaves and in the amyloplast of seeds, pulses and tubers (Ellis and other 1998). Starch contains two polysaccharide fractions, linear amylose and branched amylopectin glucose polymers (Wasserman & other 2007). Digestion of starch is dependent on how accessible the glucose chains are within the food source or the process in which they are bound together. This results in different starch sources classified as rapidly digested, slowly digested, or resistant to digestion. Freshly cooked starchy food tends to be digested more rapidly and raw cereals tend to be digested slowly (wheat, barley, oats, corn, sorghum). Those starches which escape digestion are called resistant starches. Starch digestion can be disrupted by high moisture (which increases the breakdown of the protein structure of the grain and disrupts the formation of crystalline structures), grinding (which increases surface area allowing microbes to attach), gelatinization (destruction of the crystalline structure of starch granules allowing access to molecules), and chemical treatment (Betancur and Chel 1997).

2.2. Resistant Starch

The nutritional quality of starch is determined by the state of the starch. Starch properties are based upon the amount of glucose released, which is a source of energy for the body, and the time it takes for digestion to take place. Starch can be modified from rapidly digestible to indigestible, which is called resistant starch (Englyst and other 1992). Resistant starches are also referred to as dietary starches because they escape enzymatic digestion in the small intestine and are fermented by colonic microflora in the large intestine (Wasserman and other 2007) to short chain fatty acids. The effect on the body of resistant starch is similar to dietary fiber (Berry 1986). Resistant starch is used as a functional food ingredient for human nutrition because it can

reduce caloric content and has physiological effects similar to dietary fiber (Wasserman and other 2007). Resistant starch is classified into the following four categories: type I, physically inaccessible and entrapped in a cellular matrix, which are partly milled grains and seeds; type II, native granule starches, raw potato and banana starches; type III, retrograded or crystalline starches, which can be formed during different food processing methods, such as in bread and corn flakes; and type IV, chemically modified starches. (Englyst 1992, Wasserman and others 2007).

2.3. Formation of Resistant Starch

Most research for the formation of resistant starch is connected with type III because the starches' nutritional characteristics can be saved during the cooking process. Type III resistant starch is formed by thermal disruption of the granular structure of the starch in water or gelatinization of the starch and re-crystallization of amylose and amylopectin (retrogradation). Industrial production of resistant starch comes mainly from high amylose maize starches. Type III resistant starch is formed by enzymatic debranching of gelatinized starches followed by drying, extrusion or ion crystallization (addition of salts). The formation of RS is affected by several properties of starch, such as granular structure, crystallinity, amylose and amylopectin ratio, and chain length.

The granular structure of raw starch influence RS formation. Starch granules can be viewed using a scanning electron microscope (SEM). Granular size can be important for determining the digestible characteristics of the starch. During heat and moisture treatments intermolecular bonds between amylose and amylopectin molecules can form (Kawabata and other 1994). Retrogradation of starch using HCHPA (Heating Controller High Pressure Autoclave) destroys the granular structure, which appears as irregular shaped particles having a continuous sponge-like network (Escarpa and other 1996). Under SEM it was observed that the starch granule is completely destroyed by linearization (hydrolysis in diluted hydrochloric acid)

by reducing the Short A chain fraction (DP < 10) and perfection of the residual crystallites (Zhang and other 2006). In another study Zhang and others found that a longer pre-hydrolysis time reduces the particle size of native cereal starches without affecting their natural SDS properties (Zhang and other 2006). Potato and high amylose maize starch are known to be very resistant to digestion and most cereal starches are slowly digested but completely absorbed. Potato starch has a small surface to volume ratio and in its raw form is more resistant to hydrolysis which may be due to the granular structure and amylose content (Zhang and others 2006).

The crystalline structure of granules may cause them to be resistant to enzyme hydrolysis. Crystallinity can be studied by x-ray diffraction and differential scanning calorimetry to observe chain fragments packed into the structure (Zhang and others 2006). Potato starch has a type-B crystalline structure which is more related to RS formation and cereal starches have type-A related to slowly digestible properties (Zhang and others 2006). Gelatinization of the starch eliminates the crystalline structure allowing for enzyme hydrolysis and reduces the RS content of the starch. Recrystallization and chemical modification will increase the RS content. (Zhang and others 2006).

Amylose and amylopectin ratio affects the formation of RS based on a synergistic effect between the starch molecules that affects starch hydrolysis. Retrogradation of amylopectin decreased the hydrolysis index and could not be linked to RS content. Retrograding amylose was the main factor influencing RS (Leeman and other 2006). RS formation usually increases as amylose content increases. The higher the amylose content the lower the digestibility of the starch or the larger the RS yield (Escarpa and other 1996).

Retrogradation is suggested to be the major mechanism behind the reduction in digestibility (Tovar and other 1996). Amylose is retrograded when heated with water to around 50°C allowing the amylose granule to swell (Tovar and other 1996). Amylopectin crystalline

structure begins to swell causing it to disintegrate and the granule is ruptured. The starch begins to swell due to an increased randomization of polysaccharide chains as the starch thickens and turns into a gel (gelatinization). Gelatinization causes the starch granule to lose its crystalline region due to heat. The crystalline region of the starch granule keeps water out and once heat is applied the crystalline region is destroyed allowing water to penetrate the granule and increase randomness within the starch structure. As heat is applied randomness continues to decrease the number and size of the crystalline regions. The temperature at which the crystalline region is lost is different depending on the type of starch. Gelatinization significantly increases the digestibility of the starch due to diffused amylose chains. Once gelatinization is complete the starch is easily digestible. When the gel is cooled or dried the starch begins to recrystallize (retrogradation). Recrystallization of the amylose molecule takes place quickly forming linear structures, while recrystallization of amylopectin takes place within several days of storage (Tovar and other 1996).

Ishiguro and others (2000) observed retrogradation of sweet potato starch gels by examining ten different sweet potato varieties. Gel hardness, percentage of leaked water and the contribution to retrogradation by amylose content and amylopectin chain length were measured. They observed that starch gels stored at 5°C for 2 hours and for one week caused an increase in gel hardness in some varieties and a decrease in others. After one month of storage all gels increased in hardness. The percentage of leaked water was different among the varieties although hardness increased in all varieties as the percentage of leaked water increased after one week of storage. The retrogradation properties of starch gel are important for determining properties of foods during storage.

The influence of chain length demonstrates the retrogradation index of starch. A higher portion of longer chains of amylopectin (around DP15) increases retrogradation while a higher proportion of extra-short chains of amylopectin (DP 10) decreases the retrogradation index of

starch (Ishiguro and others 2000). After enzyme debranching, chain length distributions of starch varieties are determined in order to evaluate the relationship between amylose content and chain length distribution to retrogradation. The amylose content was correlated with an increase in retrogradation properties. Retrogradation properities of sweet potato starch gels were increased when the amylose content was higher and extra long chains of amylopectin were present. Retrogradation is delayed in starches containing higher levels of short or medium length chains of amylopectin and a lower content of amylose. High performance anion exchange chromatography (HPAEC) was done to gather more information on the relationship of amylopectin chain length to retrogradation. Results demonstrated that starch with more short chains retrogrades slower than starch with a higher degree of polymerization (DP15) of longer chains which retrogrades faster (Ishiguro and others 2000).

Amylose content seems to be important for the formation of resistant starch. Higher levels of resistant starch are found in starches containing higher levels of amylose (Unlu and others 1998). Resistant starch is increased as retrogradation of the starch increases. Resistant starch is formed with an increased association of linear amylose and longer amylopectin starch molecules. Potato starches have A-type crystalline structures which have a lower degree of perfection that accounts for potato starches having a higher degree of short A chains (DP 5-10) (Zhang and others 2006). These small chains can be formed using several different methods such as acid hydrolysis, mechanical shear, heat (annealing), moisture, enzyme hydrolysis or a combination of those methods.

2.4. Heat, Moisture and pH Conditions

The formation of RS is affected by the water content. Increased RS formation is associated with repeated heat and moisture treatments which decreases enzyme susceptibility of the starch to alpha-amylase. High moisture and temperature can alter the crystalline structure of the starch granule and significantly lower RS.

Brumovsky and Thompson (2001) examined the production of a boiling stable granular resistant starch using high amylose maize starch. They discovered that a partial acid hydrolysis of a high amylose corn starch enhances the effects of hydrothermal treatments used to produce granular resistant starch that is stable against further hydrothermal treatment. Annealing treatments of gelatinized starch are difficult to achieve (Wasserman and other 2007). Annealing and heat-moisture treatment both increased the yield of boiling –stable granular resistant starch production. The combination of acid and heat caused a decrease in gelatinization enthalpy. Heat without acid did not decrease gelatinization enthalpy. In the same study the effect of a heat-moisture treatment was observed with and without a partial acid hydrolysis. However, heat-moisture treatment is more effective for producing boiling stable granular RS, which can be increased when followed by a partial acid hydrolysis (Brumovsky and Thompson 2001). This could be due to longer amylose and amylopectin chains of the native starch. The acid hydrolysis would prefer to attack the amorphous portion of the granule, if the chain ends were allowed to form double helices and associate this type of binding could be provided due to the hydrothermal treatments which would allow the chains to form higher ordered structures. (Brumovsky and Thompson 2001)

Chung and others (2003) studied the effects of acid hydrolysis on freeze-thawed corn starch and examined crystallinity and pasting properties. They found that as acid hydrolysis time increased the crystallinity of the freeze-thawed corn starch increased with peaks at 17, 20, and 22-23° typical of B and V-type crystallites. Thermal characteristics for freeze-thawed products after 2 hours of acid hydrolysis showed crystal melting enthalpies ranging from 150° to 170° which is attributed to the melting of amylose double helices. Endotherms below 130°, reflect the melting point of amylose-lipid complex. Amylopectin crystals melt between 40-70°C and was not observed in the thermographs. Acid hydrolysis decreased viscosity which may be due to the

increased crystallinity. Resistant starch did not significantly increase by acid hydrolysis. (Chung and other 2003)

Shin and others (2004) did an experiment to evaluate the effect of partial acid hydrolysis and heat-moisture treatment on the formation of resistant starch by autoclaving. The starch was hydrolyzed with acid for 8 hours than autoclaved and stored for 24 hours at 4°C. Resistant starch content ranged from 5.4-22.7%. Sweet potato had a higher RS value than potato starch after partial acid hydrolysis. Gelatinization parameters of the acid hydrolyzed starches showed a higher enthalpy and lower peak temperatures than those without acid hydrolysis. X-ray diffraction patterns for potato RS showed broad peaks at 15 and 25°. Sweet potato showed distinctive peaks at 5, 15, 17 and 22-25° but with more peaks at >25° with partial acid hydrolysis. Partial acid hydrolysis, autoclaving-cooling and heat-moisture treatments alter the starch structure and are effective methods for increasing the resistant starch content of tuber starches. (Shin and others 2004)

Collado and Corke (1999) did a study to determine the effect of heat-moisture treatment (HMT) at pH 10 on sweet potato starch pasting properties, gelatinization temperature, swelling volume, solubility and gel texture. Heat-moisture treatments under alkaline conditions demonstrated an increase in peak viscosity. Both peak temperature and enthalpy increased as HMT exposure times increased. The treated starch demonstrated a reduction in resilience and a shift from long stringy nature to short paste consistency, and starch treated by heat-moisture treatments under alkaline conditions showed a higher degree of liquid expulsion. (Collado and Corke 1999)

Similar results were found by Bryant and Hamaker (1997) upon mixing starch at strong basic pH. Bryant and Hamaker (1997) found that when starch is mixed with $Ca(OH)_2$ (lime) an increase in gelatinization temperature occurs, which is further increased when $Ca(OH)_2$ concentrations are increased. (Bryant and Hamaker, 1997)

2.5. Processing Conditions

Gelatinization and retrogradation processes may affect the formation of RS. High moisture and temperature can alter the crystalline structure of the starch granule and significantly lower RS. Increasing RS content can be done using processing methods to induce crystallization.

2.6. Thermal Processing

- Steam cooking

Tover and Melito (1996) observed the impact of steam-cooking, and the effect of dry heat under high-pressure on the enzymatic availability of starch in beans. After heating for 90 min high levels of available starch were found in the autoclaved and conventionally steamed samples. It seems that resistant starch formation is connected to the amount of retrograded amylose (Englyst 1992). This study concluded that steam-heating contributes to amylose retrogradation, which enhances resistant starch formation in legumes. Resistant starch content from raw seeds is about 9 to 15 times less than that of preheated seeds. The resistant starch level of whole beans steam-heated was less than levels within isolated starches. This may be because protein interacts with amylose, which modifies the polysaccharide recrystallization productivity (Tovar and others 1996).

- Autoclaving

Escorpa and others (1996) studied the formation of resistant starch using a high pressure autoclave process. The objective was to standardize the hydrothermal process in starch gelatinization using heated controlled high pressure autoclave (HCHPA). This enabled the researcher to have exact control over the temperature and pressure to allow more control of gelatinization and resistant starch formation in amlyose, amylopectin and potato starch. This experiment demonstrated that resistant starch increased when higher levels of amylose were present. This shows how important it is to control amylose during retrogradation of starch gels. In a study by Sievart and Pomeranz (1989) resistant starch yields increased with a decrease in

amount of water and resistant starch yields decreased with an increase in autoclave temperatures (around 148°C) (Sievart and Pomeranz 1989). In this experiment gelatinization using a boiling water bath yielded lower amounts of resistant starch than the HCHPA indicating that more resistant starch is formed when conditions are controlled rather than using a conventional autoclave or boiling water bath for gelatinization of the starch (Escorpa and others 1996).

In a study by Lehmann and others (2002) autoclaving and debranching were used on banana starch to observe the formation of resistant starch. In this study different enzyme concentrations were used to determine the best enzyme concentration and hydrolysis rate (time) to debranch the starch. It was demonstrated that a higher enzyme concentration was needed in order to debranch the starch. A concentration of 10.6U/g of enzyme was used for 12 hours then the starch was autoclaved. The results after 5 hours of enzyme hydrolysis (pullulanase) showed lower levels of available starch were obtained. Short chains were produced with an increase in debranching. The study also observed that resistant starch increased as amylose content increased. From this study debranching with an enzyme concentration of 10.6 U/g for 5 hours, then autoclaving to retrograde amylose, increased resistant starch content in banana starch. (Lehmann and others 2002)

- Extrusion

Unlu and Fallar (1998) examined the production of resistant starch using a twin-screw extruder, depending on starch type, citric acid addition and screw speed. This experiment demonstrated that resistant starch and dietary fiber percentage was greater when corn starch was added to corn meal than when potato starch was added. Adding citric acid to the different starch combinations also increased resistant starch levels significantly. The higher levels of resistant starch formed may be due to the higher amylose content found in corn starch. Brumovsky and Thompson (2001) found that potato starch was more solubilized during a boiling process, which lowers resistant starch and total dietary fiber (TDF) content, while corn starch was less

solubilized, which raises resistant starch and TDF content. (Bromovsky and Thompson 2001). Unlu and Faller (1998) proposed that when gelatinization is incomplete, digestion will also be incomplete resulting in a higher resistant starch and TDF content. The increase in TDF and resistant starch content formed with acid hydrolysis could have been a result of reductions in the size of starch polymers amylose and amylopectin. In the experiment of high amylose corn starch (HACS), citric acid (CA) & screw speed, HACS & CA levels increased resistant starch formation. As CA and HACS were increased and screw speed was decreased from 300 to 200 rpm, resistant starch levels increased. A high screw speed (300rpm) resulted in a decrease in resistant starch and TDF, which may have been a result of shorter residence time allowing less time for the linear amylose chains to associate. A longer residence time allowed more time for the chains to associate at the lower screw speed of 200 rpm. This experiment concluded that with increased levels of HACS, extrusion will increase resistant starch yield. (Unlu and Faller 1998)

An experiment was done to determine the optimal conditions of an extruder and its effect on forming resistant starch in pastry wheat flour (Kim and other 2006). Barrel temperatures of 40, 60, 80, 100, and 120°C were maintained as well as a feed rate of 30 g/min. The moisture content was adjusted to 20%, 40% and 60% using a water injector. After extrusion, extrudates were cut and cooled to room temperature then dried at 50°C for 16 hours, milled and stored at -20 °C until analyzed. Resistant starch content increased after extrusion on day 0 from 1.3 to 7 fold and further increased from 3 to 11 fold during 7 and 14 days of storage, respectively. Feed moisture was significant, especially at 60%, for the formation of resistant starch, which may be due to optimal moisture conditions for retrogradation. The screw speed was also significant at 250 rpm for resistant starch formation, but storage period was more important. This experiment demonstrates that resistant starch formation increases as feed moisture percentage (60%), storage time (14 days) and screw speed (250 rpm) increased. Results from DSC showed that enthalpy decreased as screw speed increased and feed moisture levels decreased. Extrusion conditions

having low feed moisture (20%) and high screw speed (250rpm) produced extrudates with a high thermal stability. Results for pasting properties by RVA for 20% feed moisture levels showed that pasting decreased with an increase in screw speed. In 40% feed moisture samples, peak and breakdown increased significantly while trough, final viscosity and setback properties decreased as screw speed increased. Pasting properties of 60% feed moisture (FM) samples had significantly higher peak, trough, final viscosity and setback than other tested samples. (Kim and others 2006)

2.7. Storage Conditions

RS content increases during storage at low temperatures. In a study on the effect of storage on the retrogradation of sweet potato starch, starch gels were evaluated on their degree of gel hardness and percentage of leaked water. After one month of storage at room temperature starch gels were highly retrograded (Ishiguro and others 2000).

2.8. Resistant Starch Determination

- In vitro

Englyst and others (1992) determined the percentage of undigested raw potato starch and total digested starch after 120 min of alpha-amylase hydrolysis. Results showed that boiled potato, corn and wheat starches were completely digested after two hours of hydrolysis and the native corn and wheat starch was 30-40% undigested which demonstrates the amount of type II resistant starches in the native starches. This demonstrates that boiling removes type II resistant starch from potato, wheat and corn. (Englyst and others 1992)

- In vivo

The *in vivo* method provided data for determining rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS). This method compared results from analytical techniques to results from healthy ileostomates. This study by Englyst et al. (1996) was designed to yield values of RS which are defined as the sum of starch and starch degradation that reached

the human large intestine (Englyst and others 1992). The analytical data based on this definition of RS was shown to accurately predict the amount of starch that is likely to escape complete digestion and absorption into the human small intestine (Englyst and others 1996).

2.9. Slowly Digestible Starch

Slowly Digestible Starch (SDS) defined by Engyst is the amount of starch that is likely to be completely digested in the small intestine between 20 and 100 min (Englyst 1992). SDS can be used to physiologically benefit individuals with type 2 diabetes because it prevents hyperglycemia and hypoglycemia. It can also be used in food products that assist in weight loss and can be beneficial to athletes by providing a longer consistent source of systemic glucose (Wolf and others 1999).

A study was done to research potential starch ingredients that may be used as a source of slowly digested starch in liquid external formulas (Wolf and others 1999). Starch digestion was observed by an *in vitro* method for chemically modified starches and compared to unmodified controls. Starches were evaluated for etherification (substitution with propylene oxide), cross-linking with phosphorous oxychloride (intermolecular bridges between starches), dextrinization (acid modification with heat) and oxidation with sodium hypochlorite. The first step evaluated the extent of digestion at 15 hours of incubation, which determines the amount of starch that escapes digestion in the small intestine. The results were that unmodified waxy and dull waxy starch contained high levels of digestible starch and the 50% amylose starch contained high levels of resistant starch. Total starch was decreased in the samples treated with propylene oxide so digestibility decreased. It was found that digestibility decreased in starches as the degree of dextrinization increased and digestibility was not affected by starch modification during cross-linking. In cooked 5-10% starch in water solutions, the starch became digestible. The lab references raw corn and raw potato starch had high levels of total starch and potato starch had the highest level of resistant starch. After an *in vitro* digestion method, corn syrup solids were

rapidly and completely digested. Potato buds were rapidly digested and raw corn starch was slowly digested over time. The second experiment was done to evaluate the digestion rate of starch hydrolysis over time. An incubation period of 15 hours was correlated with the amount of starch not digested in the small intestine, so incubation was evaluated up to 15 hours. Results showed that generally the starch was digested within 2.5 hours of incubation. Raw corn starch was the only starch that demonstrated a slow rate of digestion from 67% within 2.5 hours to 86% after 15 hours. A third experiment evaluated the digestion rate from 0 to 2.5 hours. Again none of the modified starch ingredients appeared to have a slow digestion rate compared to the lab references. Raw corn starch was 35% digested in 0.5 hours and 64% digested in 2.5 hours (Wolf and others 1999).

In a study by Zhang and others (2006), native cereal starches were examined to determine their natural ability for slow digestion based on the starch granule properties. Semicrystalline structure was critical to SDS properties, which were lost during cooking. A-type cereal starches have SDS properties while B-type potato starches have resistant starch properties. Results showed that SDS properties of native cereal starches were decreased and RDS was increased by cooking in a boiling water bath for 20 min for rice, wheat, waxy maize and potato starches. Cooking completely destroys the semicrystalline structure of the native starch granules making the crystalline structure critical for SDS properties. A-type starches (normal/waxy maize, rice and wheat) have very similar chain length profiles. B-type starches like potato starch have a large percentage of long chains compared to A-type starches. Amylopectin is the molecule that forms the crystallites in starch granules and the structure of the starch is dependent upon the organization and type of starch crystalline structure. The structure of amylopectin, especially the short chain fraction which forms the crystalline region, is necessary in order for starch to have SDS properties. In the cereal starches tested, all contained a large portion of short chain links having the highest SDS properties compared to the potato starch with a fewer short chains and

more long chains less resembling SDS. These results support their previous prediction that SDS properties are determined by the crystalline region of the starch granule. (Zhang and others 2006)

Chemical modifications were done in order to produce a modified food starch high in SDS. Four starches were chemically modified by crosslinking and stabilization reactions, the starch treated by esterification with OSA (2-octen-1-ylsuccine anhydride) increased both SDS and RS. Treatment with dry heat at 130°C increased the SDS content and decreased RS content. The dry heat changed the physical and digestion characteristics of the starch. When OSA starch was heated the pasting temperatures decreased and peak viscosities increased. Dry heating also decreased To, Tp, Tc and gelatinization enthalpy (ΔH) in the unmodified waxy corn, but increased these parameters in the OSA-waxy corn and heated OSA-waxy corn. (Han and BeMiller 2007)

In order for SDS to form in starch, the starch crystallites must melt and then recrystallize. It is important to determine the temperature at which the starch crystallites melt. Rice starch was adjusted to 20% moisture and heated in a DSC to 140°C to determine optimum parameters. Starches were heated to gelatinization temperatures then held for 60 min. Digestibility decreased by 25% in non-waxy rice starch and 10% in waxy rice starch. Other rice samples did not show a significant decrease in digestibility. The waxy rice starches were heated in a microwave and conventional oven to gelatinization temperatures. Results showed a slight but significant increase in digestibility. Digestibility was higher when starch was heated for 30 min then 60 min. The non-waxy rice starch digestibility was not significantly different than control. However the heat-moisture treatment held at the melting temperature in the DSC was significant for forming SDS. (Anderson and others 2002)

2.10. Sweet Potato

Sweet potatoes are native to the tropical parts of the Americas, and were domesticated there at least 5000 years ago (CIP, 2006). The many varieties of sweet potatoes (Ipomoea

batatas) are members of the morning glory family, Convolvulacea. There are two basic types of sweet potatoes grown in the U.S., Moist-flesh (soft) and Dry-flesh (firm) types. The skin color can range from white to yellow, red, purple or brown. The flesh also ranges in color from white to yellow, orange, or orange-red. When cooked, those in the 'firm' category remain firm, while 'soft' varieties become soft and moist. It is the 'soft' varieties that are often labeled as yams in the United States. Today the U.S. Department of Agriculture requires labels with the term 'yam' to be accompanied by the term 'sweet potato' (Lucier and others 2002).

Sweet potaoes are sources of beta carotene, vitamin C, niacin, riboflavin, thiamin and minerals (Zuraida 2003). Orange-fleshed sweet potato (OFSP) is particularly promising because its levels of provitamin A carotenoids are high and can easily be absorbed by the body. Sweet potato is considered an excellent food security crop in sub-Saharan Africa because it often survives when other crops (for example, maize) fail. It is also is less labour intensive than most other staple crops, is produced using vines instead of seeds, and can be planted over a broad range of time without considerable yield loss. But most varieties in Africa are white-fleshed, lacking in beta-carotene, the precursor of vitamin A. (Lucier and others 2007)

Roots, tubers-cassava, potato, sweet potato, and yam demonstrate a significant role in the global food system. They contribute to the energy and nutrition requirements of more than 2 billion people in developing countries and will continue to do so over the next two decades. They are produced and consumed by many of the world's poorest and most food-insecure households. Roots and tubers also constitute an important source of employment and income in rural, and often marginal, areas, and for women. Moreover, they adapt to a wide range of uses: food security crop, regular food crop (consumed in fresh or processed form), cash crop, feed crop, and raw material for industrial uses. Cassava, potato, and sweet potato rank among the top 10 food crops produced in developing countries. (Scott and others 2000)

The U.S. is the 10th largest producer of sweet potatoes (Lucier and others 2002). The Frenchmen who established the first settlement at Opelousas in 1760 discovered the native Attakapas, Alabama, Choctaw, and Opelousas Indian Tribes eating sweet potatoes. The sweet potato became a favorite food item of the French and Spanish settlers and thus continued a long history of cultivation in Louisiana. In 1987, at the Louisiana State University Agricultural Center (LSU AgCenter) Larry Ralston developed the Beauregard, a new variety of high-quality, high-yield sweet potato. The Beauregard is now the dominant variety grown by the Louisiana's 300 sweet potato farmers on a collective 23,000 acres. Louisiana's sweet potato industry revenues are about $105 million, and Louisiana accounts for about 24 percent of the nation's sweet potato crop, second only to North Carolina, which claims about 40 percent of industry sales. Louisiana sweet potatoes account for 57 percent of the vegetable cash receipts at $46 million (Lucier and others 2002)

The Evangeline sweet potato released by the LSU AgCenter in 2007 is a new variety of sweet potato developed by Dr Don LaBonte. The Evangeline sweet potato variety, Ipomoea batatas (L.) Lam., demonstrates superior disease resistance to southern root-knot nematode, has a dark orange flesh that is high in sucrose content compared to the Beauregard sweet potato. The Evangeline sweet potato was grown commercially in 2008 and has a better taste that the Beauregard sweet potato. (LaBonte and others 2008)

2.11. Effect of Protein and Amino Acids on Starch Properties

Starch has many useful applications which allow it to be widely used in the food industry. Starch contains a high energy value for consumers and can provide many useful physical properties during processing. Starch can be used as a thickener, stabilizer and bulking agent based upon its gelling ability. Proteins affect the gelatinization of starch by forming complexes with starch molecules on the granule surface, and preventing the escape of exudates from the granules, thereby increasing the gelatinization temperature of the starch (Olkku and Rha 1978).

Starch can also provide certain desirable digestible characteristics based upon its regrogradation potential and other functional properties. Starches are known through research to be enhanced by the addition of amino acids (An and King 2009). An and King (2009) found that in ozonated starch when lysine was added the starch had increased in cooking stability, had higher swelling properties, and was easier to cook. Hamaker and Griffin (1993) studied the effect of protein on rice starch and found that protein can alter the gelatinization and pasting characteristics of starch.

Liang (2003) studied the effects of various amino acids on pasting characteristics, gelatinization, and X-ray diffraction pattern on rice starch and found that amino acids increased the rate of starch swelling, resulting in lower pasting viscosities and lower cooking stability. He also found that positively charged and negatively charged amino acids had a stronger influence on starch pasting than neutral amino acids. Charged amino acids increased the crystallinity of the starch, which would potentially enhance the resistant starch.

Lockwood and others (2008) studied the effects of amino acids on pasting and thermal characteristics of white and orange-fleshed Beauregard sweet potato starch. They found that charged amino acids altered pasting characteristics more than neutral amino acids and lysine allowed for more stability during cooking when added to orange-flesh sweet potato starch.

CHAPTER 3. EFFECTS OF pH TREATMENT AND AMINO ACID ADDITIVES ON GELATINIZATION CHARACTERISTICS OF SWEET POTATO STARCHES USING DIFFERENTIAL SCANNING CALORIMETRY (DCS)

3.1. Introduction

Differential scanning calorimetry (DSC) is a thermal technique that measures the amount of heat required to increase the temperature of a sample. DSC measures both temperature and enthalpies of gelatinization. DCS records phase transitions such as melting, glass transitions or exothermic decompositions which involve a change in energy or heat capacity changes. (Fennema and other 1996)

Starch is not soluble in water but when heat is applied with a sufficient amount of water, the glass transition temperature is reached and the starch granules begin to swell allowing water to penetrate into the starch granule. The starch begins to solubilize in the water, increasing the viscosity of the starch and creating a viscous paste. (Fennema and other 1996)

Data collected from the DSC analysis is presented in joules/gram (J/g °C). The collected data from the starch gelatinization parameters are recorded as peak onset, peak temperature, end of peak and gelatinization enthalpy information. Different varieties of the same species can have variation in gelatinization temperatures. Lockwood and King (2008) found apparent differences in gelatinization characteristics between white-fleshed and orange-fleshed sweet potato starches. The orange-fleshed sweet potato starch granules gelatinized at a lower temperature than those of the white-fleshed sweet potato starch.

Amino acids have been found to alter starch gelatinization characteristics. Liang (2001) found that rice starch gelatinization parameters were increased with the addition of charged amino acids including aspartic acid and lysine (Liang 2001). An (2005) found that lysine when added to rice starch increased gelatinization characteristics, onset temperature, peak temperature and conclusion temperature, while the total enthalpy used to gelatinize the starch decreased. Lockwood (2008) found that orange-fleshed sweet potato starch was mostly affected by the

addition of lysine, which increased the gelatinization temperature. The white-fleshed sweet potato starch was affected by lysine and aspartic acid. Both amino acids increase the gelatinization temperature (Lockwood 2008).

Sweet potatoes were used in this research because of the global availability and ability to grow under a variety of climate conditions. Sweet potatoes are an excellent source of starch and used based upon a continuation of previous research.

The objectives of this study were 1) to determine the effect different positively charged amino acids would have on the thermal properties of sweet potato starches and 2) to determine if altering the pH of the amino acids in a solution would affect their binding ability and therefore alter thermal properties of sweet potato starch and 3) to investigate the differences between orange-fleshed Beauregard and Evangeline sweet potatoes by use of DSC.

3.2. Materials and Methods

3.2.1. Materials

Sweet potato starch was extracted from sweet potatoes harvested in September 2008 by the Louisiana State University AgCenter research station. Both Evangeline and Beauregard sweet potatoes were used for this study. Amino acids (arginine, lysine and histidine) used in this study were purchased from Sigma Chemical Company (St. Louis Missouri). The amino acids used included positively charged Arginine, Lysine and Histidine. These amino acids were chosen based upon past research (Liang, 2001 and Lockwood, 2008). pH solutions were prepared by adjusting distilled water with NaOH and HCl to obtain pH values of 3 and 10.

3.2.2. Sweet Potato Starch Extraction

Evangeline and Beauregard sweet potatoes were washed, peeled and sliced. Then in batches of 400 grams the sliced sweet potatoes were blended at high speed for 2 min. in a Waring Blender with 500 mL of distilled water. The resulting mixture was then passed through a 150 µm sieve. The pulp on top of the sieve was washed with another 500 mL of distilled water.

Three batches were combined before the next step. The filtrate (approximately 3000 mL) was divided equally between four 800 mL centrifuge bottles. These bottles were then centrifuged at 3000 x g at 2°C for 10 min in a Thermo Electronic Corporation Sorvall RC 6 Plus Centrifuge (Waltham, MA) along with a Sorvall SLC-4000 Super-Lite rotor. Then, the liquid was discarded and the orange layer manually scraped off the starch, using a spatula. Bottles were refilled with 500 mL of distilled water to resuspend the starch, and centrifuged in the same manner. Each batch was centrifuged and washed with distilled water four times. Once centrifugation was complete the precipitate (starch) was removed from the bottle, frozen at -80 °C, and freeze dried to a fine powder. All batched were combined and stored in hermetically sealed plastic bags to form a uniform sample. This same process was repeated for each variety of sweet potato tested. The sweet potatoes yielded between 30 and 50 grams of starch per 400 g of sweet potato.

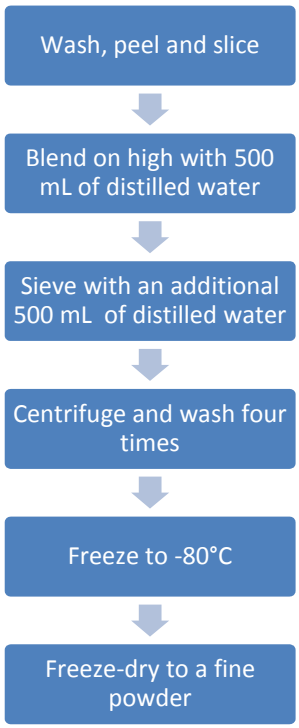

Figure 3.1. Starch extraction flowchart

3.2.3. Starch Treatment

Amino acids arginine, lysine and histidine (all positive charged) were added on a 6% dry weight starch basis to both Beauregard and Evangeline sweet potato starch. 1M HCL and/or 1M NaOH was used to adjust 500 mL of distilled water to obtain a pH solution of either 3 or 10. The pH solutions were added to the starch and starch-amino acid mixtures on a 1:4 starch water ratio. For freeze-dried samples, solutions were mixed using magnetic stirring bars at 30 and 60 min intervals. Solutions were placed into freeze-drying trays and frozen to -80 °C, freeze-dried and placed into an airtight container. For oven-dried samples the solutions were mixed using magnetic stirring bars and placed into an oven pre-set at 40 °C until dried to a powder (~4-6 hours). Once the oven-dried samples have been dried, the samples were ground to a fine powder using a mortar and pestle and stored in an airtight container. Starch samples prepared include starch and amino acid (arginine, lysine or histidine); starch and pH solution (3 and 10) and starch-amino acids with pH solution mixture. Controls were made with just water addition.

3.2.4. Moisture Content

The moisture content was determined by placing an aluminum dish in the oven (to tare) at 130°C (~1hour). The aluminum dish was removed and placed it into a desiccator for 2 min until room temperature. The aluminum dish was placed on a scale and the weight recorded. The scale was tarred and 2 grams of prepared starch sample was placed into the aluminum dish. The aluminum dish containing the sample was placed into the preheated oven for 1 hour. After 1 hour the aluminum dish containing the sample was removed and placed into the desiccators to cool (about 2 min). The aluminum dish containing the sample was weighed and the weight recorded. Total solids from residue and moisture were determined. (AOAC 925.10)

3.2.5. Proximate Analysis

Native Beauregard and Evangeline sweet potato starches were examined for lipid content using chloroform methanol (method 983.23, AOAC 1995), protein content using thermal

conductivity on a Model 2410 Nitrogen Analyzer (Perkin Elmer, Norwalk, CT) (method 992.15, AOAC 1995), ash content using a Phoenix Microwave ashing System (CEM, Matthews, NC) (method 920.153, AOAC 1995), and moisture content using a SMART System 5 (CEM, Matthews, NC) (method 985.14, AOAC 1995). The carbohydrate content was determined by using the formula: 100-(% protein + % fat + % moisture + % ash) = % carbohydrate. Trace metal content of the native sweet potato starches were quantified through the use of ICP (Inductively Coupled Plasma). Each starch sample was analyzed in duplicate. The replicates were then averaged.

3.2.6. Amylose Content Determination

Amylose content of the native sweet potato starch samples was done following the Megazyme Amylose Assay Procedure (Megazyme International, Ireland). All reagent solutions, buffers and solvents were prepared beforehand following the instructions given by Megazyme. The analyses were performed in duplicate. Twenty to twenty-five mg of starch samples were accurately weighed into 10mL screw capped tubes. One mL of DMSO was added to the tubes and mixed on low speed on a vortex mixer (about 10 seconds). The tubes were capped and heated in a boiling water bath, to dissolve the sample. The contents of the sealed tubes were vigorously mixed at high speed on a vortex mixer, after which the tubes were placed in a boiling water bath and heated for 15 min. with intermittent high-speed stirring on a vortex mixer. The tubes were then stored at room temperature for 5 min. and 2 mL of 95% ethanol was added with continuous stirring on a vortex mixer. A further 4mL of ethanol was added; the tubes were capped and inverted to mix. The tubes were allowed to stand for 15 min. at room temperature to allow a starch precipitate to form. The tubes were centrifuged at 2000 x g for 5 min. the supernatant discarded and the tubes were drained on tissue paper for 10 min. ensuring that all of the ethanol had drained. The starch pellet was used in the subsequent amylose and starch determinations. Two mL of DMSO were added to the starch pellets. The tubes were place

in a boiling water bath for 15 min. and mixed occasionally. On removing the tubes form the boiling water bath, 4 mL of Concanavalin A solvent (30 mL of a 600 mM, pH 6.4 sodium acetate buffer diluted to 100 mL with distilled water) were immediately added, the tubes were mixed thoroughly and then the tube contents were quantitatively transferred to 25 mL volumetric flasks. The contents were diluted to volume with Concanavalin A solvent, this mixture is Solution A. One mL of Solution A, from the above section, was transferred to a 2.0 mL Eppendorf microfuge tube, 0.5 mL of Concanavalin A solution (200 mg ConA, a lectin protein, in 50 mL ConA solvent) was added, then the tubes were capped and gently mixed by repeated inversion. The tubes were allowed to stand for 1 hour at room temperature, and then centrifuged at 14,000 x g for 10 min in a microfuge at room temperature. One mL of the supernatant was transferred to 15 mL centrifuge tubes. Three mL of 100 mM sodium acetate buffer, pH 4.5 were then added. This reduced the pH to 5. The contents were mixed; the tubes were lightly stoppered and heated in a boiling water bath for 5 min to denature the Con A. The tubes were placed in a water bath at 40°C and allowed to equilibrate for 5 min, then 0.1 mL of amyloglucosidase (3300 U/mL)/α-amylase (500 U/mL) enzyme mixture was added and the tubes were incubated at 40 °C for 30 min. The tubes were centrifuged at 2000 x g for 5 min. To 1.0 mL aliquots of the supernatant, 4 mL of GOPOD Reagent [glucose oxidase (>12,000 U/mL) plus peroxidase (>650 U/mL) and 4-aminoantipyrine (80 mg) diluted in 20 mL of GOPOD Reagent Buffer (potassium phosphate buffer (1 M, pH 4.7), p-hydroxybenzoic acid (0.22 M) and sodium azide (0.02 % w/w)] was added. The tubes were then incubated at 40 °C for 20 min. A Reagent Blank was made by adding 1.0 mL of 100 mM sodium acetate buffer to 4.0 mL of GOPOD Reagent; the D-Glucose Controls were made by adding 0.1 mL of D-glucose standard solution (1 mg/mL) and 0.9 mL of sodium acetate buffer to 4.0 mL of GOPOD reagent. The Reagent Blank and the D-Glucose Controls were incubated concurrently with the 25 starch samples. The absorbance of each sample and the D-glucose controls were read at 510 nm against the reagent blank.

Figure 3.2. Amylose procedure

3.2.7. Differential Scanning Calorimeter (DSC) Analysis

A Differential Scanning Calorimeter (DSC) Q10 (TA Instrument, New Castle, DE) was used to determine the gelatinization properties of the sweet potato starch samples. Ten mg of starch sample was weighed and placed into the steel DSC pans. Twenty microliters of distilled water was added and the lids were sealed with a rubber o-ring. A pan with 20 µL of water was used as a reference. The temperature was run from 35 °C to 140°C at 5°C/ minute. Afterwards,

the DCS thermographs were analyzed to identify any tendency relating to amino acid additive or pH adjustment. All DCS analyses were performed in duplicate.

3.2.8. Statistical Analysis

SAS (Statistical Analysis System) software (version 9.1) was used to analyze the DSC data. Standard deviation, ANOVA (Analysis of Variance), and Tukey's Studentized Range (HSD) were used to examine the effects of the amino acid additives on the thermal properties of sweet potato starches, on a $p \leq 0.05$ level. The abbreviations used were Arg for arginine, Lys for lysine and His for histidine.

3.3. Results and Discussion

3.3.1. Proximate Analysis and Amylose Content

The results from the proximate analysis on both sweet potato starches are shown in Table 3.1. The Beauregard sweet potato starch had a higher fat, fiber and moisture content than Evangeline starch. Both contained a very small amount of protein.

Table 3.1. Proximate Analysis Results

	Beauregard	**Evangeline**
Analyte	**%**	**%**
Protein	0.161±0.0	0.242±0.0
Crude Fat	0.22±0.3	0.105±0.1
Crude Fiber	0.06±0.1	0±0.0
Moisture	3.97±0.7	1.83±0.4
Ash	0.08±0.1	0.07±0.1
Amylose	23.6±1.2	27.1±0.3

The Beauregard sweet potato yielded a starch with 23.6% amylose, while the Evangeline sweet potato starch contained 27.1% amylose, (Table 3.1). Amylose content for native

Beauregard starch was 23.6 and native Evangeline starch was 27.1. These two amylose values were somewhat different. Moorthy (2002) found that sweet potatoes have an amylose content around 20 %.

Table 3.2. Trace Mineral Analysis Results

	Beauregard	**Evangeline**
Elements	Results	Results
Aluminum, (ppm)	N.D.	1.37±0.0
Boron, (ppm)	N.D.	N.D.
Calcium (%)	0.01±0.0	0.02±0.0
Copper, (ppm)	1.33±0.0	N.D.
Iron, (ppm)	N.D.	N.D.
Magnesium (%)	N.D.	N.D.
Manganese, (ppm)	N.D.	N.D.
Molybdenum, (ppm)	N.D.	N.D.
Phosphorous, (ppm)	N.D.	N.D.
Potassium (%)	0.01±0.0	0.01±0.0
Sodium, (ppm)	10.47±0.0	9.88±0.0
Sulfur (%)	N.D.	N.D.
Zinc, (ppm)	1.57±0.0	1.8±0.0

*N.D. means no detectable limit

Table 3.2 shows the trace mineral analysis for native Beauregard and Evangeline sweet potato starch. Evangeline had a higher aluminum content compared to the Beauregard starch while Beauregard was higher than the Evangeline starch in copper. Both native Beauregard and Evangeline starches had somewhat similar detectable limits for calcium, potassium, sodium and zinc.

3.3.2. Differential Scanning Calorimeter Analysis

- Beauregard Sweet Potato Starch Thermal Properties

For freeze-dried Beauregard sweet potato starch, the onset temperature was not significantly affected by pH or amino acid treatments compared to native starch (Table 3.3). Peak temperature significantly decreased at pH 3 for 30 min and one hour. A decrease in gelatinization temperature was found at pH 10 alone and pH 10 with histidine for 30 min compared to native and control.

Table 3.3. Beauregard Freeze-dried DSC Results [1,2,3]

Treatment	pH	Time	Onset temp (°C)	Peak Temp (°C)	Conclusion Temp (°C)	Enthalpy (J/g°C)
Native			57.1±0.85 abc	74.1±0.24ab	84.1±0.21 ab	8.9±0.42 a
control	no	0min	46.8±1.28 c	73.5±0.16 abc	85.0±0.04 ab	14.4±0.35 a
Arg	no	0min	56.6±4.82 abc	75.1±0.64 a	86.8±0.55 ab	9.7±3.57 a
Lys	no	0min	50.4±0.69 bc	75.2±0.49 a	84.3±0.49 ab	13.4±0.73 a
His	no	0min	55.4±3.8 abc	73.7±0.18 abc	81.3±1.42 b	7.2±3.1 a
noaa	3	30min	55.6±7.8abc	70.6±0.47 efghi	84.1±0.02 ab	11.5±4.85 a
Arg	3	30min	55.5±4.12 abc	72.2±0.28 bcdefg	86.5±2.26 ab	12.9±2.18 a
Lys	3	30min	59.8±2.23 ab	72.9±0.75 bcd	88.8±4.72 a	12.6±3.10 a
His	3	30min	58.3±2.83 abc	70.5±0.05 fghi	81.5±2.51 ab	11.1±1.97 a
noaa	10	30min	62.0±2.45 ab	71.4±0.24 defgh	83.0±0.88 ab	7.8±2.63 a
Arg	10	30min	63.4±1.22 a	72.5±0.0 bcde	83.6±0.47 ab	8.9±1.07 a
Lys	10	30min	60.6±1.43 ab	72.1±0.06 bcdefg	82.9±2.05 ab	10.8±1.65 a
His	10	30min	60.7±0.41ab	70.8±0.33 efgh	82.5±2.0 ab	9.0±0.63 a
noaa	3	1hour	60.3±5.1 ab	70.7±0.22 efghi	82.8±1.91 ab	10.2±4.7 a
Arg	3	1hour	60.4±4.6 ab	72.1±0.02 cdefg	83.7±4.00 ab	10.7±3.39 a
Lys	3	1hour	63.9±2.16 a	72.3±1.11 bcdef	81.9±1.85 ab	7.8±1.23 a
His	3	1hour	59.6±1.27 ab	70.2±0.47 ghi	81.3±0.32 b	9.9±1.63 a
noaa	10	1hour	56.6±1.46 abc	68.7±0.21 i	80.4±0.69 b	11.9±0.87 a
Arg	10	1hour	63.1±1.12 a	71.4±0.54 defgh	81.7±0.34 ab	8.9±0.01 a
Lys	10	1hour	58.7±0.44 abc	71.2±0.98 defgh	83.1±0.25 ab	12.5±0.99 a
His	10	1hour	58.7±1.16 abc	69.7±0.79 hi	81.2±1.42 b	10.7±0.11 a

[1]Means in the same column with the same letter are not significantly different at $p \geq 0.05$
[2]Control is native starch with water added prior to specify drying method.
[3]Arg is arginine, Lys is lysine and His is histidine

Lai and others (2004) studied the effect of alkalizing agents on nonwaxy and waxy starches. Lai and others (2004) found that gelatinization temperatures decreased with NaOH and Na_2CO_3

treatments for nonwaxy wheat, corn, and rice starch. After one hour of pH 10 treatment a decrease in peak temperature was found at pH 10, with and without arginine, lysine and histidine compared to native and control (Table 3.3, Figure 3.3).These results were opposite to findings from An (2005) where it was found that lysine increased the gelatinization temperatures of both ozone treated and non-ozone treated rice starch samples. Ito and others (2004) found that charged amino acids both positive and negative, increased the gelatinization temperatures of potato starch. Lockwood (2005) found that lysine increased the gelatinization temperature. Ito and others (2004) added amino acids on a 10 % starch basis while this study added amino acids on a 6 % starch basis. The results from this study correspond with previous studies that positive charged amino acids along with pH treatments can alter gelatinization temperature of sweet potato starch. There was no significant difference between pH treatments of 30 min and one hour, except for pH 10 alone. No differences were observed for conclusion temperature and enthalpy (Table 3.3).

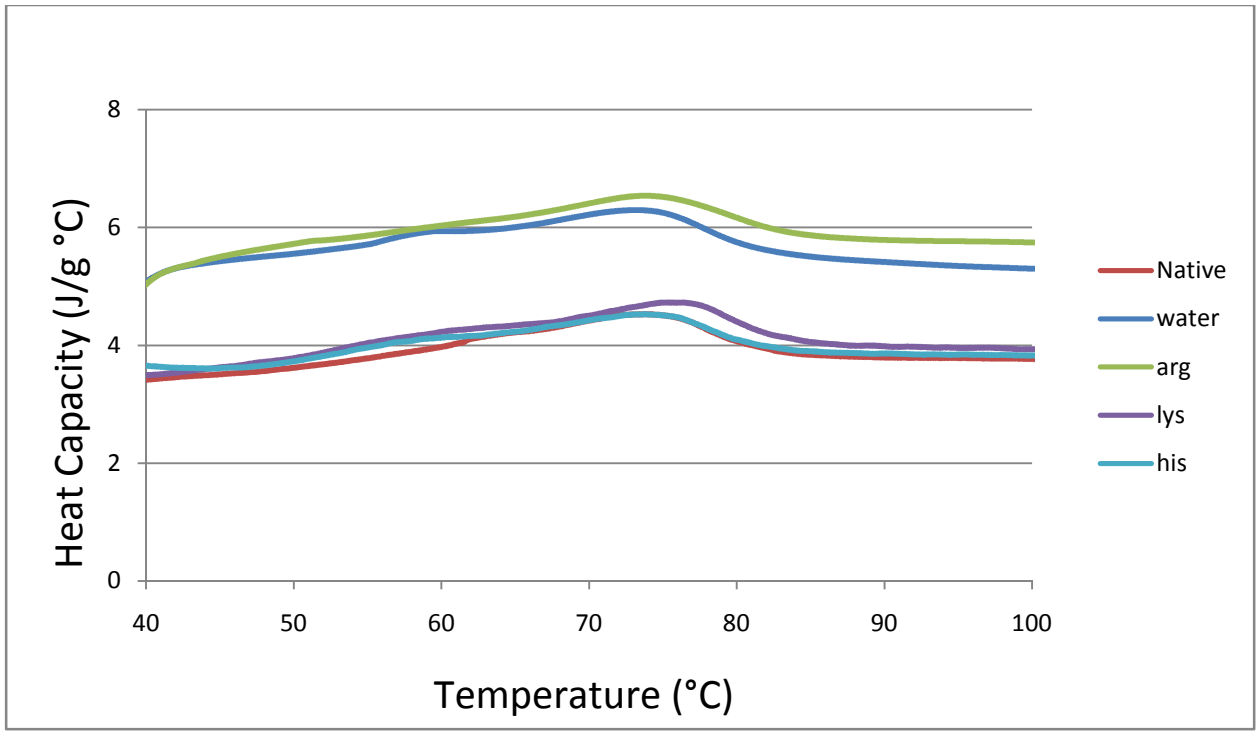

Figure 3.3. DSC thermogram of Freeze-dried Beauregard Sweet Potato Starch with Amino Acids alone

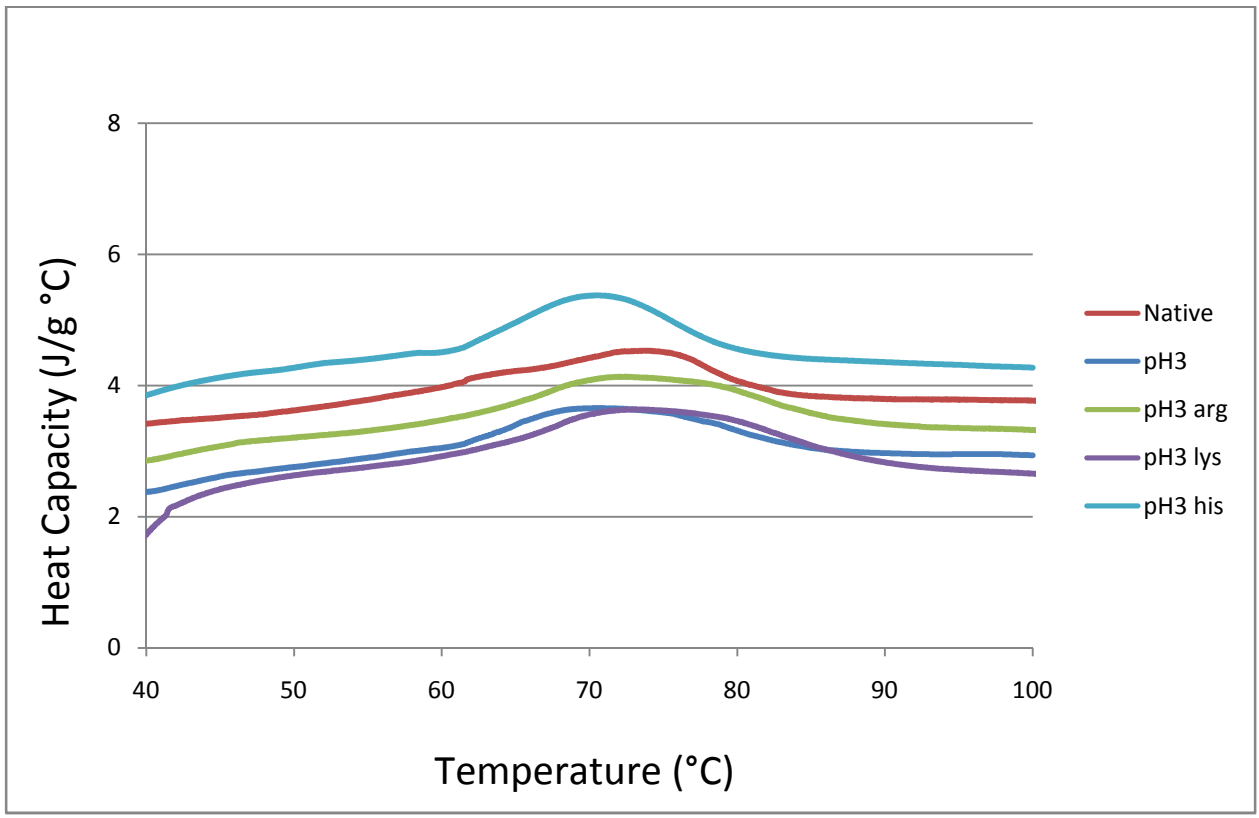

Figure 3.4. DSC thermogram of Freeze-dried Beauregard Sweet Potato Starch with Amino Acids at pH3 for 30 min

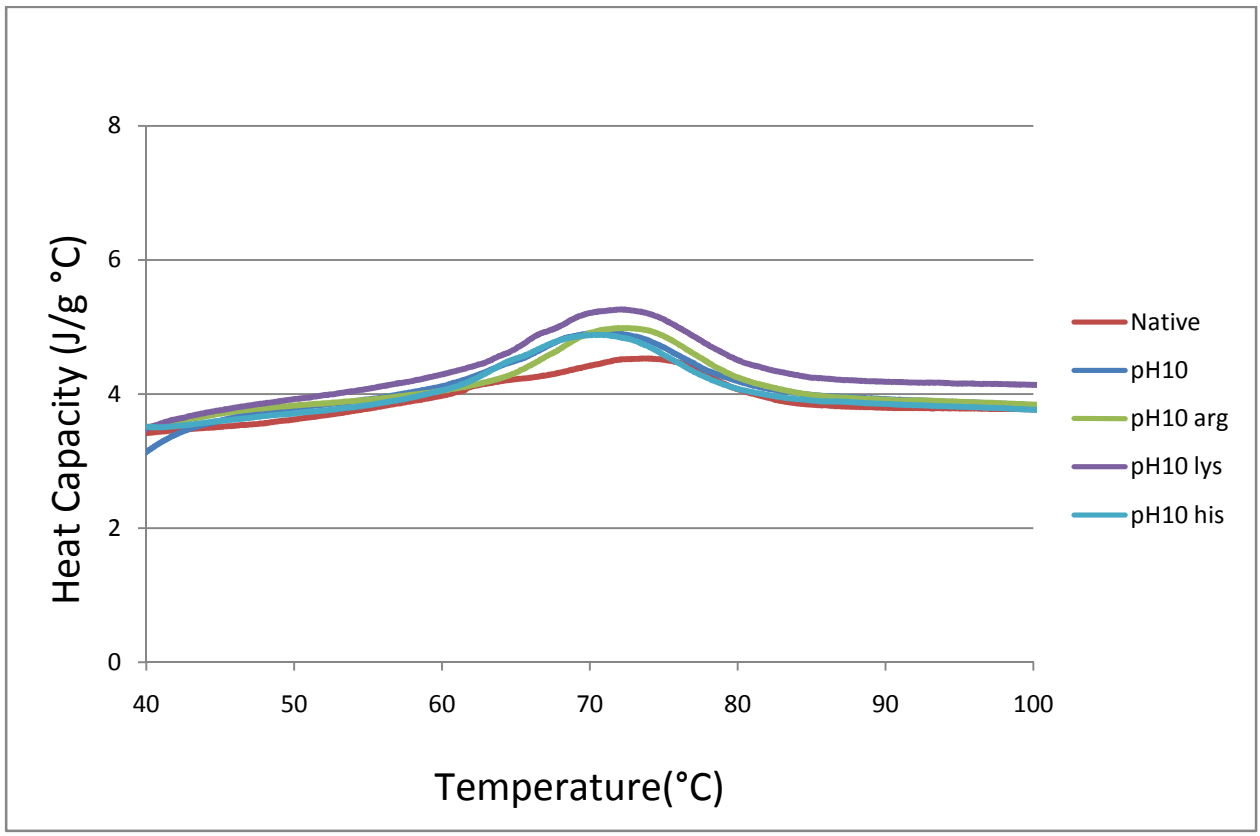

Figure 3.5. DSC thermogram of Freeze-dried Beauregard Sweet Potato Starch with Amino Acids at pH10 for 30 min

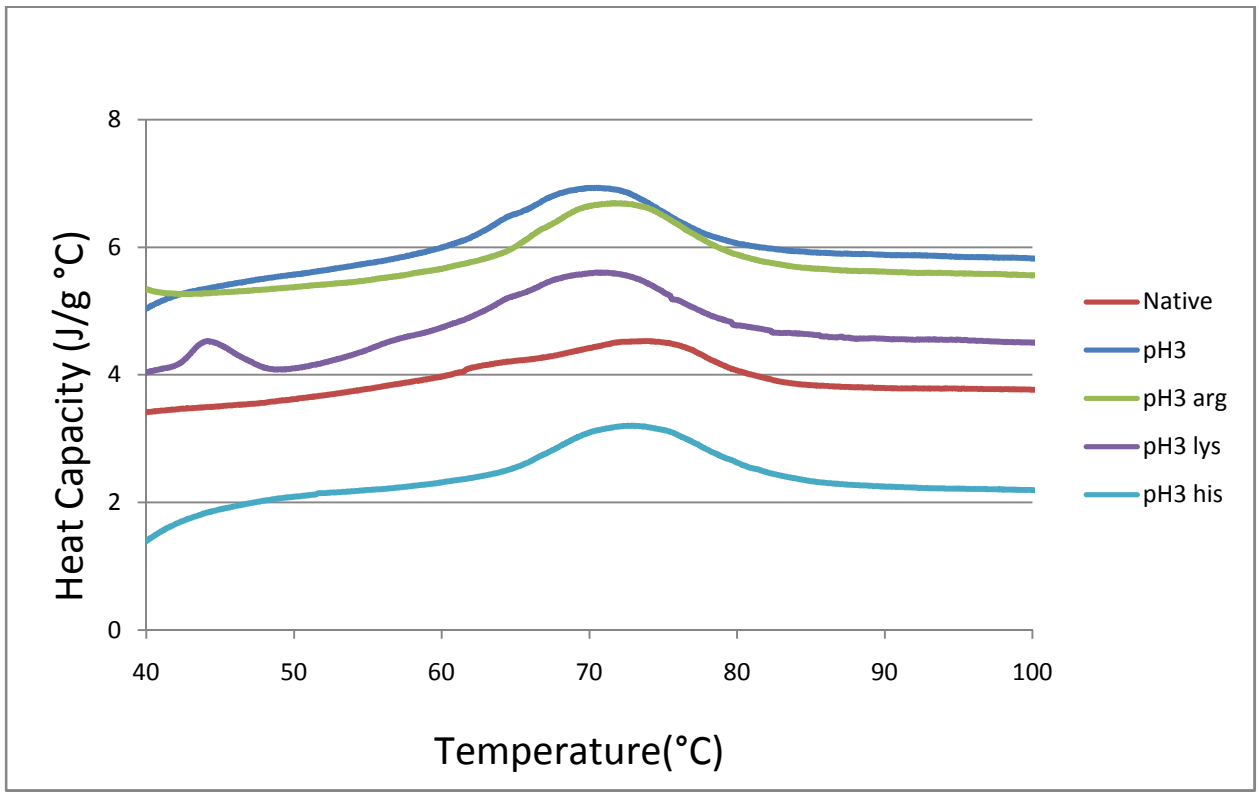

Figure 3.6. DSC thermogram of Freeze-dried Beauregard Sweet Potato Starch with Amino Acids at pH3 for 1 hour

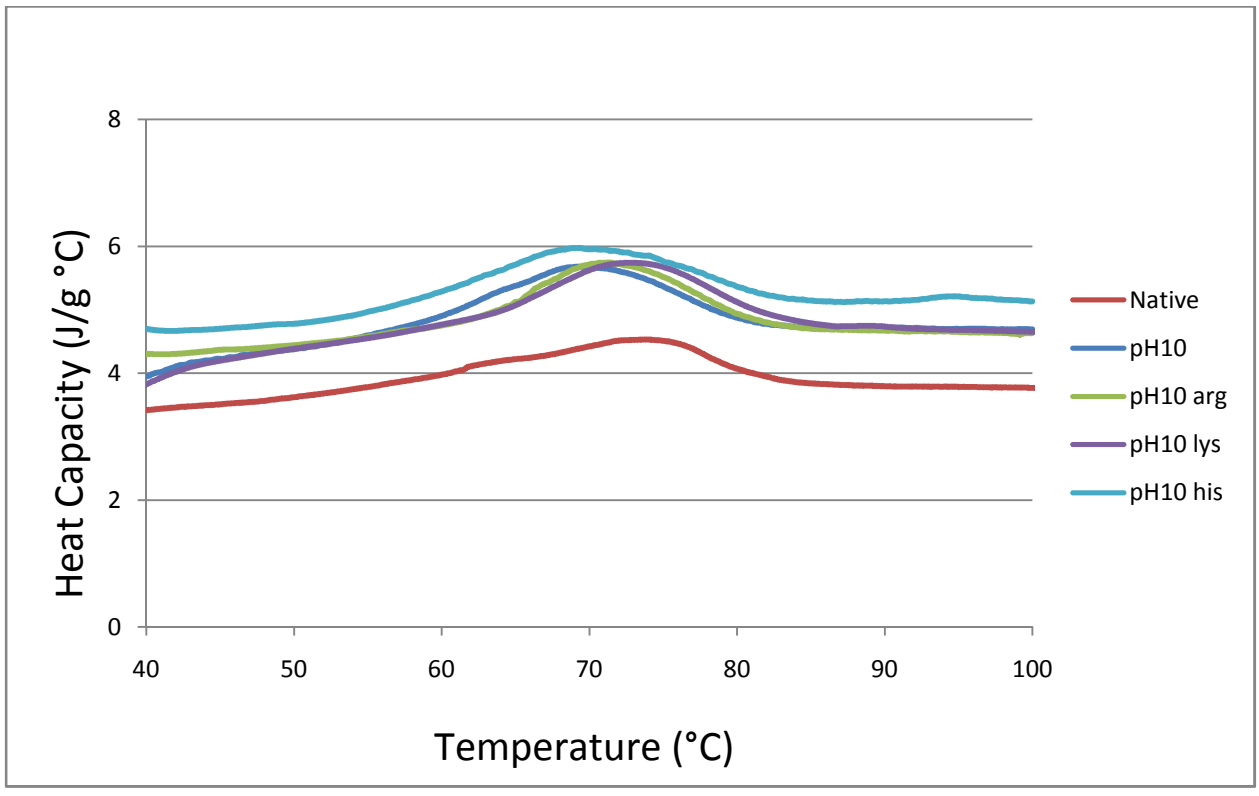

Figure 3.7. DSC thermogram of Freeze-dried Beauregard Sweet Potato Starch with Amino Acids at pH10 for 1 hour

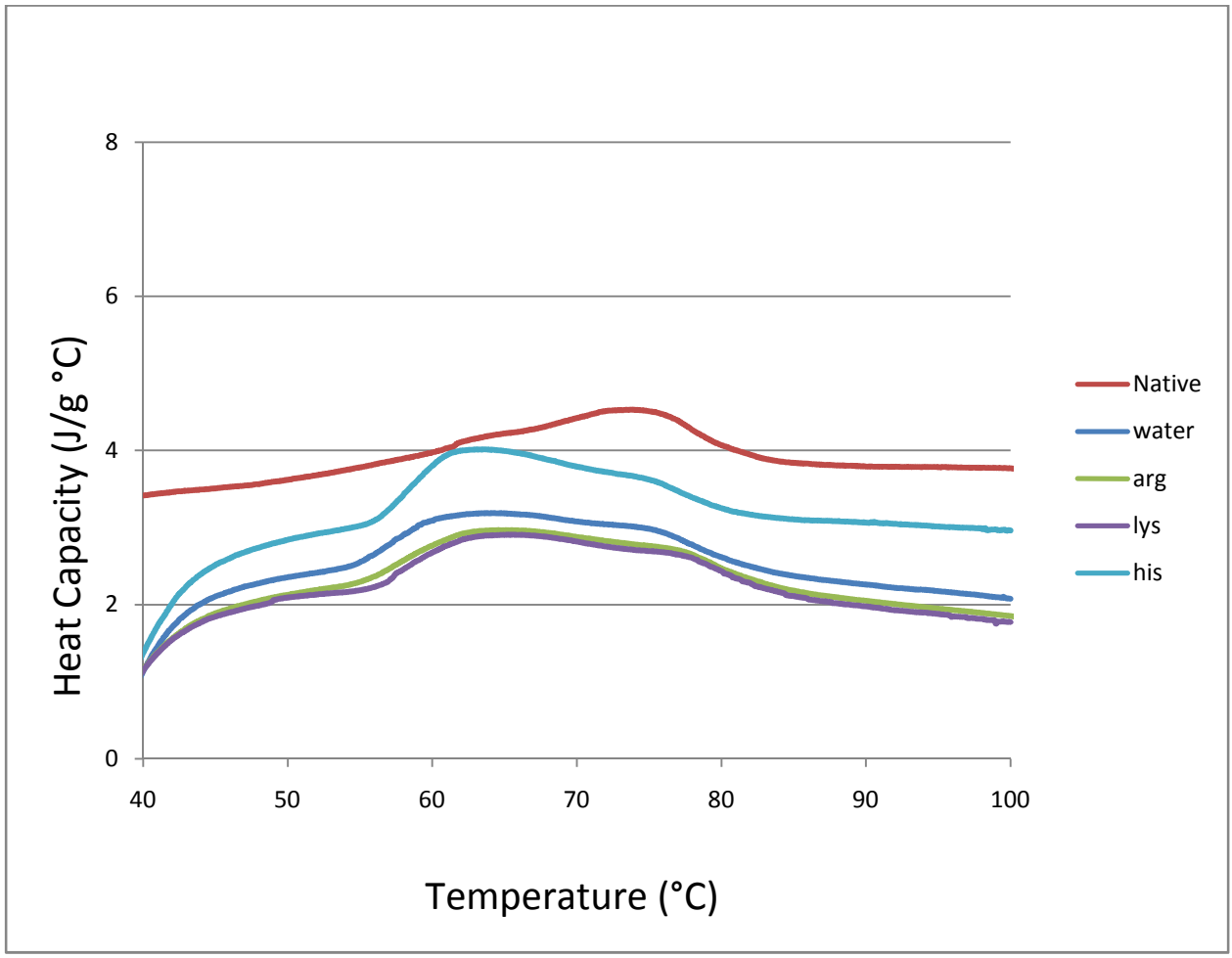

Figure 3.8. DSC thermogram of Oven-dried Beauregard Sweet Potato Starch with Amino Acids alone

For oven-dried Beauregard sweet potato starch, the onset temperature was not significantly changed by the addition of amino acids or pH treatments. (Table 3.4) The gelatinization temperature significantly decreased from the native starch (74.1 °C) with the addition of amino acids of arginine, lysine and histidine but were not significantly different from control (Table 3.4). Lockwood and King (2008) studied the effects of amino acid additives on thermal properties of starch and found that lysine increased gelatinization temperature of orange-fleshed sweet potato starch. pH 3 alone decreased the peak temperature below the native starch but was higher than the control (Table 3.4, Figure 3.7). Lysine and histidine with pH 3 decreased the peak temperature below that of the native and above that of the control starch significantly

but was not different from pH 3 alone. These findings are different from Thirathumthavorn and Charoenrein (2005) who studied the thermal properties of acid treated rice starch and found that gelatinization temperatures increased with longer hydrolysis time (up to 48 hours). The addition of pH 10 solution and lysine at pH 10 significantly decreased the gelatinization temperature below the native starch but above the control, but there was no difference between pH 10 alone or pH 10 with amino acids. Lockwood (2005) found that charged amino acids altered gelatinization properties of sweet potato starch. Orange-fleshed sweet potato starch granules gelatinized at lower temperatures (67.69 °C) compared to white fleshed sweet potato starch and lysine increased the gelatinization temperature to (70.89 °C).

Table 3.4. Beauregard Oven-dried DSC Results [1,2,3]

Treatment	pH	Time	Onset temp (°C)	Peak Temp (°C)	Conclusion Temp (°C)	Enthalpy (J/g°C)
Native			57.1±0.85a	74.1±0.24a	84.1±0.21a	8.93±0.42b
Control	no	0min	54.2±0.39a	64.7±0.34de	84.5±1.22a	14.1±0.54ab
Arg	no	0min	55.2±1.88a	66.1±0.24d	85.4±2.35a	13.5±1.21ab
Lys	no	0min	56.4±0.71a	66.2±0.33d	84.9±2.11a	13.2±1.9ab
His	no	0min	55.7±0.06a	64.3±1.39e	83.8±1.51a	13.8±1.12ab
noaa	3	30min	59.7±0.01a	71.0±0.2bc	83.3±1.48a	10.9±0.01ab
Arg	3	30min	59.4±2.33a	72.7±0.05ab	86.5±4.81a	13.2±2.72ab
Lys	3	30min	56.8±2.2a	71.9±0.16bc	84.9±0.37a	14.8±1.66ab
His	3	30min	58.6±1.12a	70.8±0.5c	83.5±0.69a	13.4±0.86ab
noaa	10	30min	57.2±1.36a	70.9±0.13bc	83.9±1.79a	14.6±1.22ab
Arg	10	30min	56.5±2.43a	72.5±0.05abc	85.1±3.11a	15.7±2.63a
Lys	10	30min	55.9±1.65a	71.9±0.11bc	85.2±1.32a	15.2±1.39a
His	10	30min	57.5±0.57a	70.9±0.23dc	83.0±0.69a	14.2±1.01ab

[1]Means in the same column with the same letter are not significantly different at $p \geq 0.05$
[2]Control is native starch with water added prior to specify drying method.
[3]Arg is arginine, Lys is lysine and His is histidine

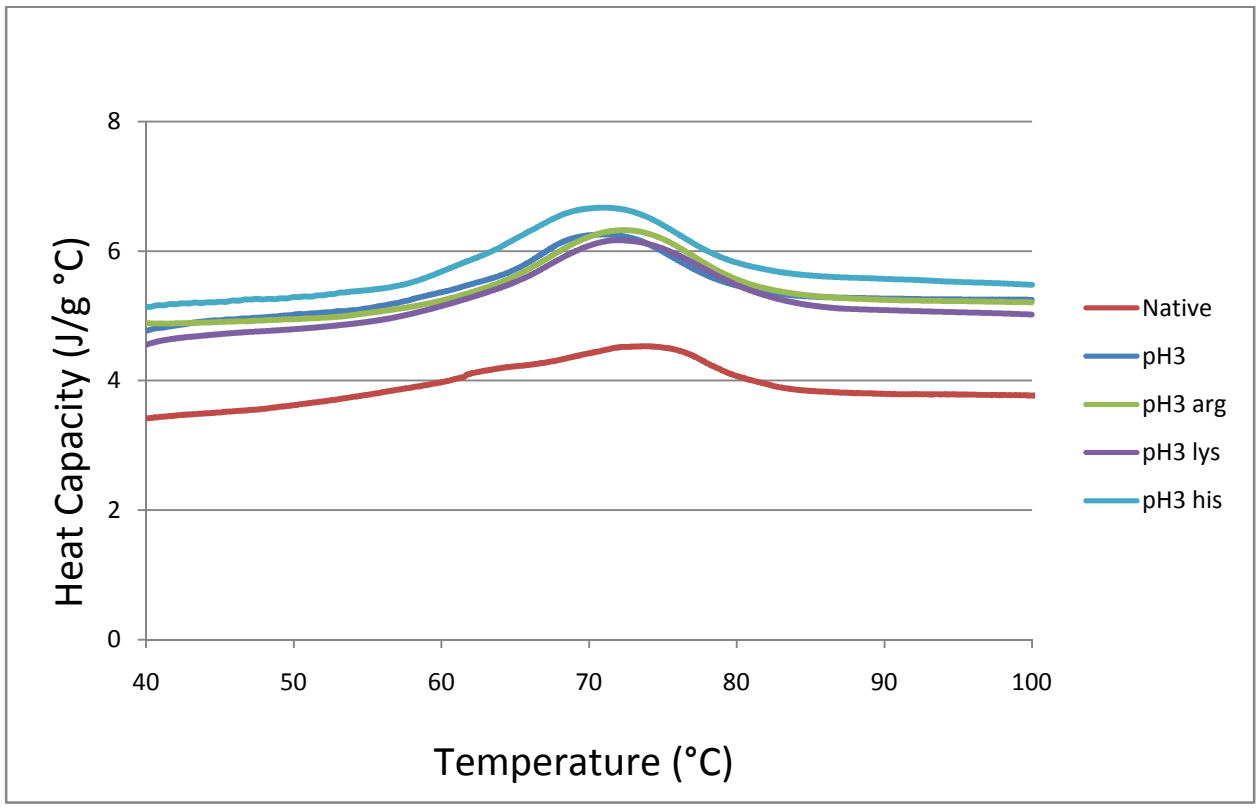

Figure 3.9. DSC thermogram of Oven-dried Beauregard Sweet Potato Starch with Amino Acids at pH3

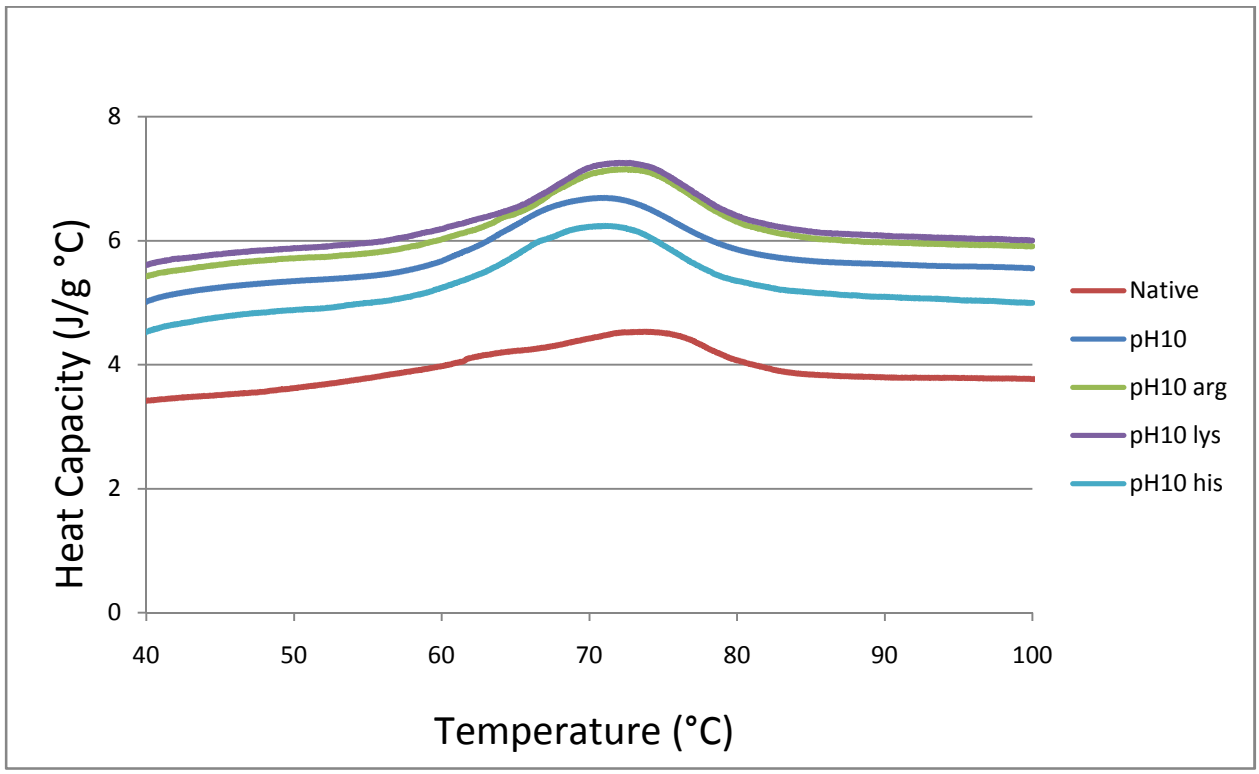

Figure 3.10. DSC thermogram of Oven-dried Beauregard Sweet Potato Starch with Amino Acids at pH10

- Evangeline Sweet Potato Starch Thermal Properties

For freeze-dried Evangeline sweet potato starch, the onset temperature was not significantly altered by the addition of amino acids and/or pH treatments (Table 3.5). The peak temperature decreased from the native starch with the addition of pH3 for 30 min and histidine with pH3, but were not different from control. There were no significant changes in conclusion temperature or enthalpy. There was no significant difference between pH treatments of 30 min and pH treatments of one hour (Table 3.5).

Table 3.5. Evangeline Freeze-dried DSC Results [1,2,3]

Treatments	pH	Time	Onset temp (°C)	Peak Temp (°C)	Conclusion Temp (°C)	Enthalpy (J/g°C)
Native			66.8±2.06a	75.5±0.48abcd	84.9±2.16 a	4.3±1.65bcd
control	no	0min	64.5±0.25a	74.3±0.53bcdefg	81.9±0.38a	4.7±0.72bcd
Arg	no	0min	69.4±0.26a	75.8±0.09ab	82.4±0.53a	2.8±0.21d
Lys	no	0min	69.0±0.89a	76.6±0.18a	83.2±0.13a	3.1±0.25d
His	no	0min	66.4±1.18a	75.1±0.13abcdef	83.0±0.33a	3.4±0.51cd
noaa	3	30min	64.4±0.74a	73.7±0.0g	82.7±0.0a	8.2±0.33abcd
Arg	3	30min	60.9±1.25a	74.8±0.16abcdef	85.0±0.0a	11.4±0.33abcd
Lys	3	30min	59.6±5.66a	75.2±0.07abcdef	85.9±3.42a	11.9±3.17abc
His	3	30min	55.7±0.38a	73.3±0.2fg	84.6±1.46a	13.7±1.27a
noaa	10	30min	60.2±3.97a	73.5±0.21defg	82.7±0.42a	10.7±2.31abcd
Arg	10	30min	57.2±9.23a	74.2±0.13bcdefg	84.9±1.45a	12.3±4.23ab
Lys	10	30min	65.9±0.11a	74.9±0.01abcdef	84.8±2.37a	8.5±0.77abcd
His	10	30min	61.12±4.33a	73.4±0.06efg	83.9±0.51a	10.2±2.91abcd
noaa	3	1hour	63.1±3.53a	74.2±0.51bcdefg	83.1±0.72a	8.8±2.21abcd
Arg	3	1hour	58.4±8.69a	74.8±0.71abcdef	85.1±1.63a	12.1±4.11abc
Lys	3	1hour	66.4±0.1a	75.0±0.34abcdef	82.7±1.53a	7.3±1.01abcd
His	3	1hour	59.6±3.12a	73.3±0.21fg	82.3±2.39a	9.9±2.08abcd
noaa	10	1hour	62.3±3.33a	73.7±0.02bcdefg	82.9±2.04a	9.3±2.9abcd
Arg	10	1hour	67.3±0.2a	75.4±0.05abcde	86.6±4.23a	8.1±0.99abcd
Lys	10	1hour	64.7±2.0a	75.7±0.26abc	84.8±1.7a	8.8±1.76abcd
His	10	1hour	55.2±7.12a	73.2±0.08fg	85.4±2.1a	13.9±3.14a

[1]Means in the same column with the same letter are not significantly different at $p \geq 0.05$
[2]Control is native starch with water added prior to specify drying method.
[3]Arg is arginine, Lys is lysine and His is histidine

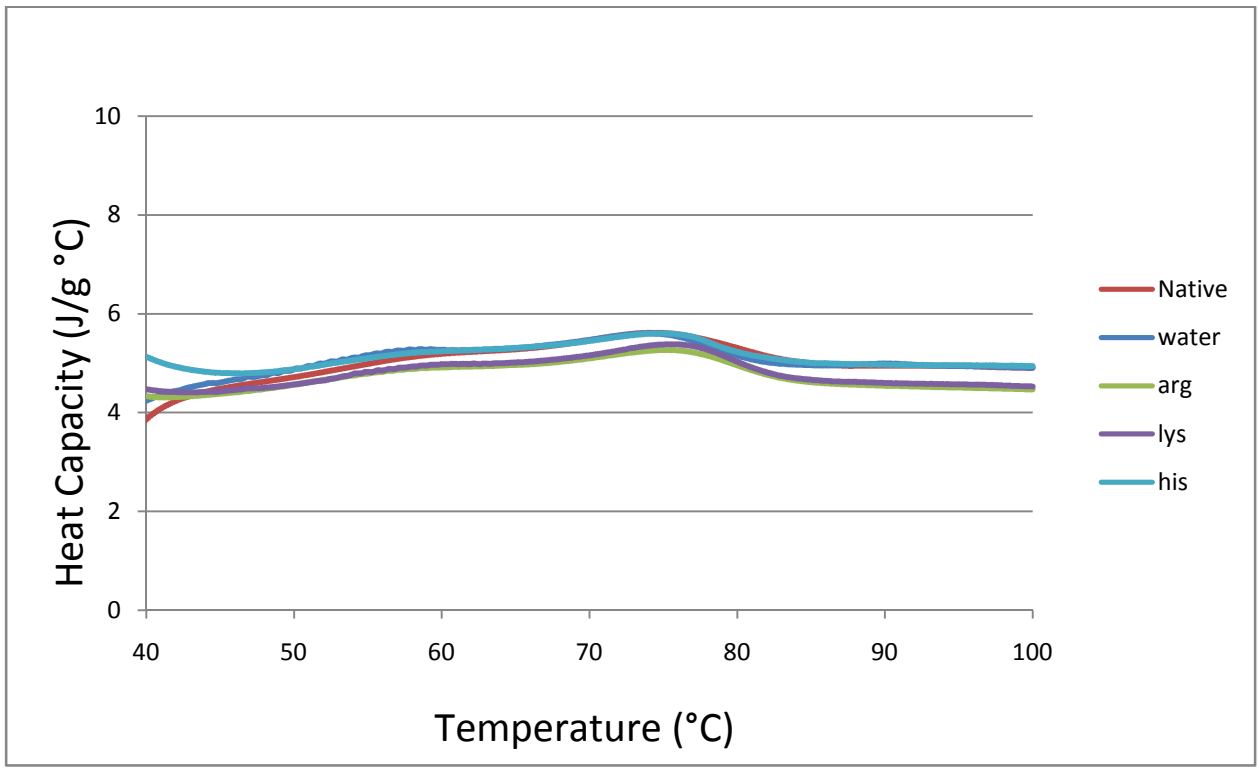

Figure 3.11. DSC thermogram of Freeze-dried Evangeline Sweet Potato Starch with Amino Acids alone

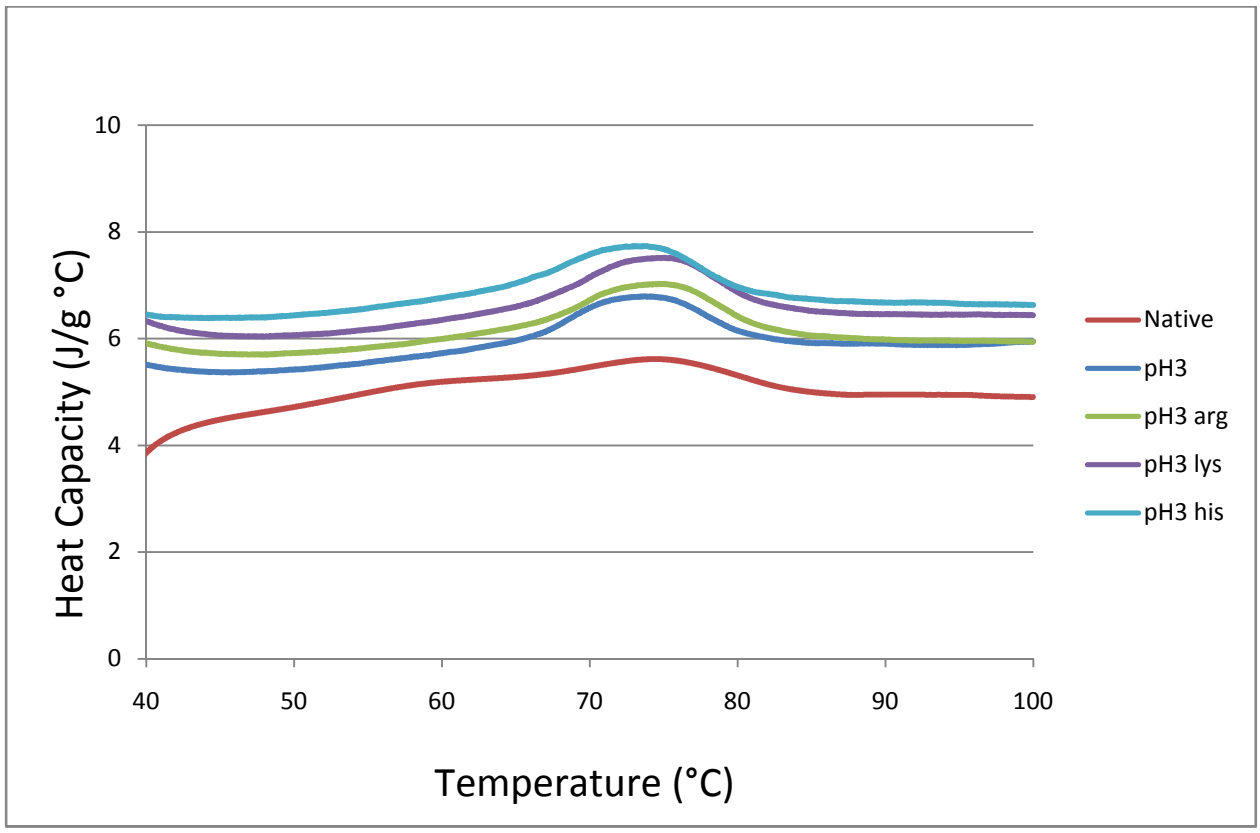

Figure 3.12. DSC thermogram of Freeze-dried Evangeline Sweet Potato Starch with Amino Acids at pH3 for 30 min

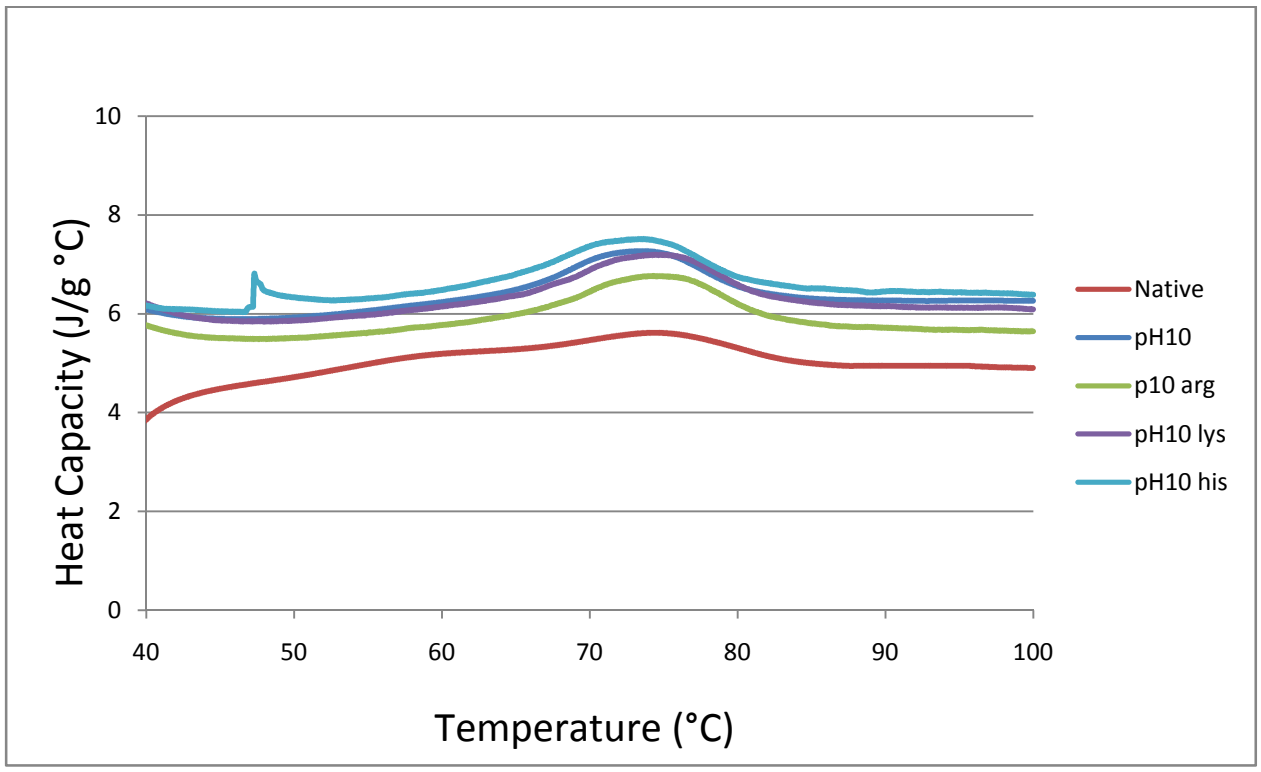

Figure 3.13. DSC thermogram of Freeze-dried Evangeline Sweet Potato Starch with Amino Acids at pH10 for 30 min

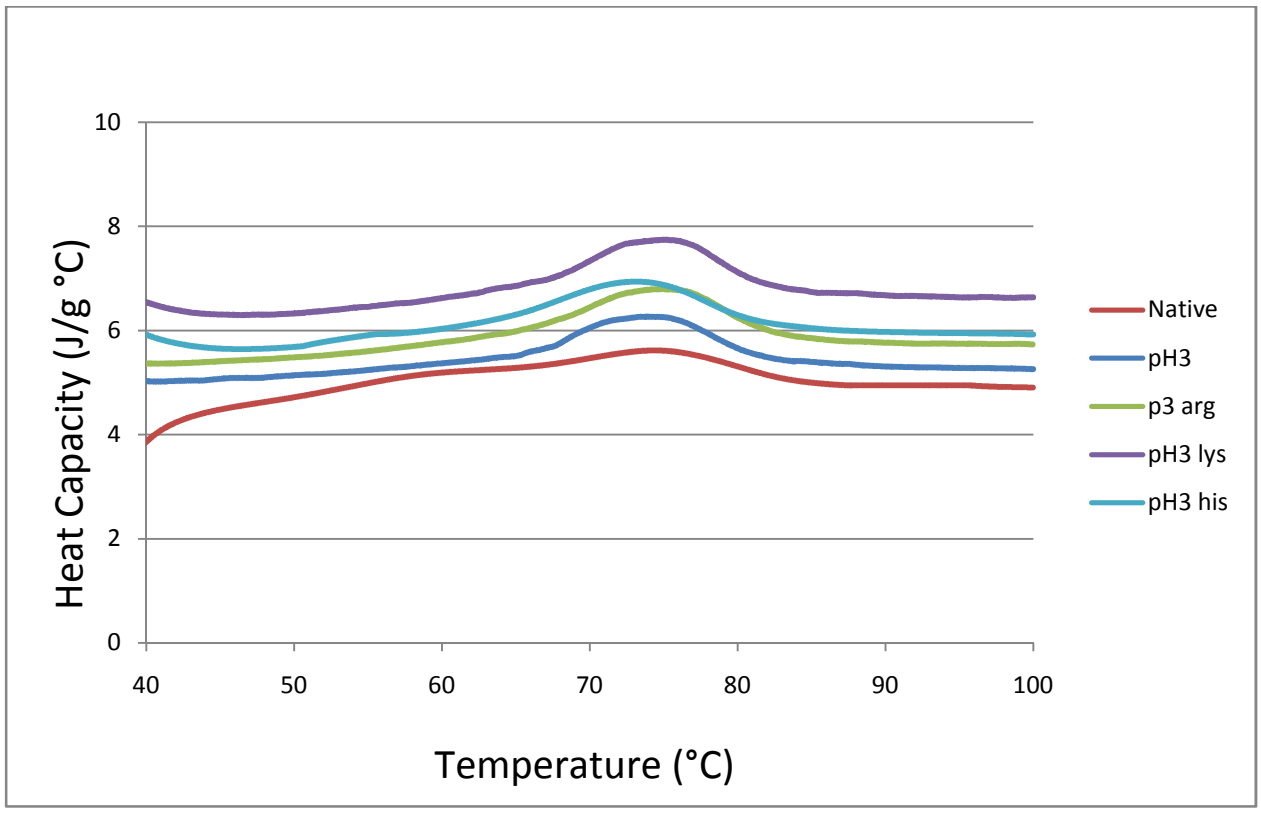

Figure 3.14. DSC thermogram of Freeze-dried Evangeline Sweet Potato Starch with Amino Acids at pH3 for 1 hour

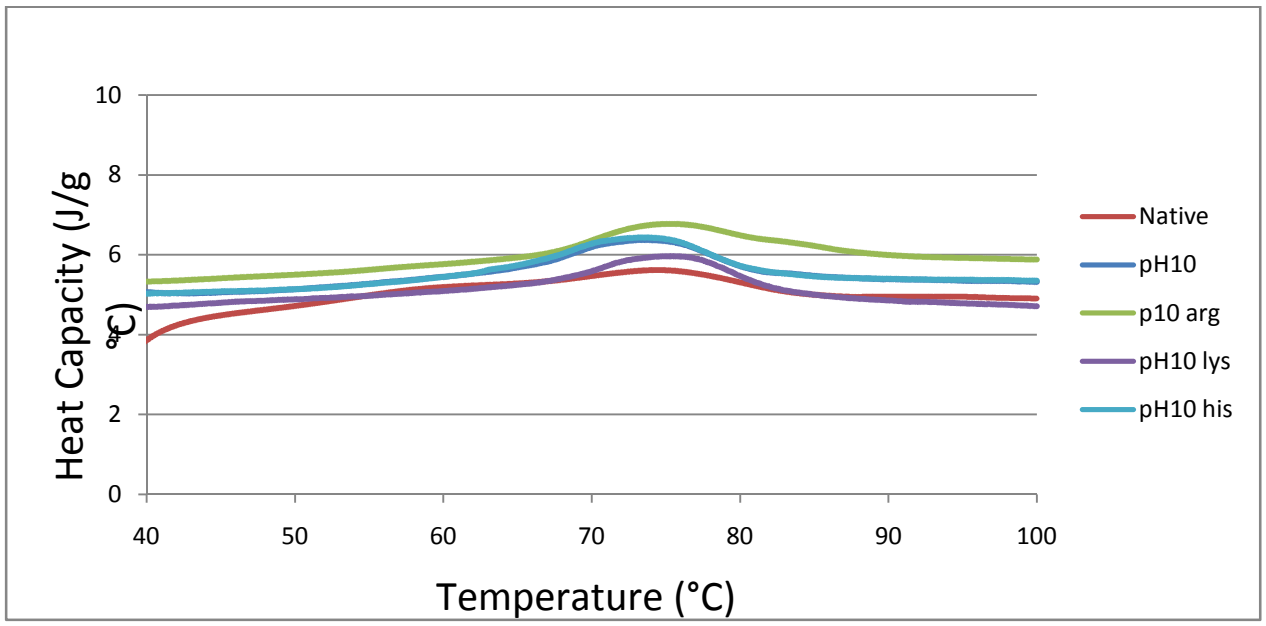

Figure 3.15. DSC thermogram of Freeze-dried Evangeline Sweet Potato Starch with Amino Acids at pH10 for 1 hour

For oven-dried Evangeline sweet potato starch, the onset temperature was significantly decreased from the native starch with the addition of amino acids and pH treatments but treated samples were not different from control (Table 3.6). The peak temperature was significantly decreased from the native starch and control with the addition of histidine at pH 3 and pH 10 but was not significantly different from pH treatment alone (Figure 3.5, Figure 3.6). Conclusion temperatures and enthalpies were not affected by pH or amino acid treatments (Table 3.6).

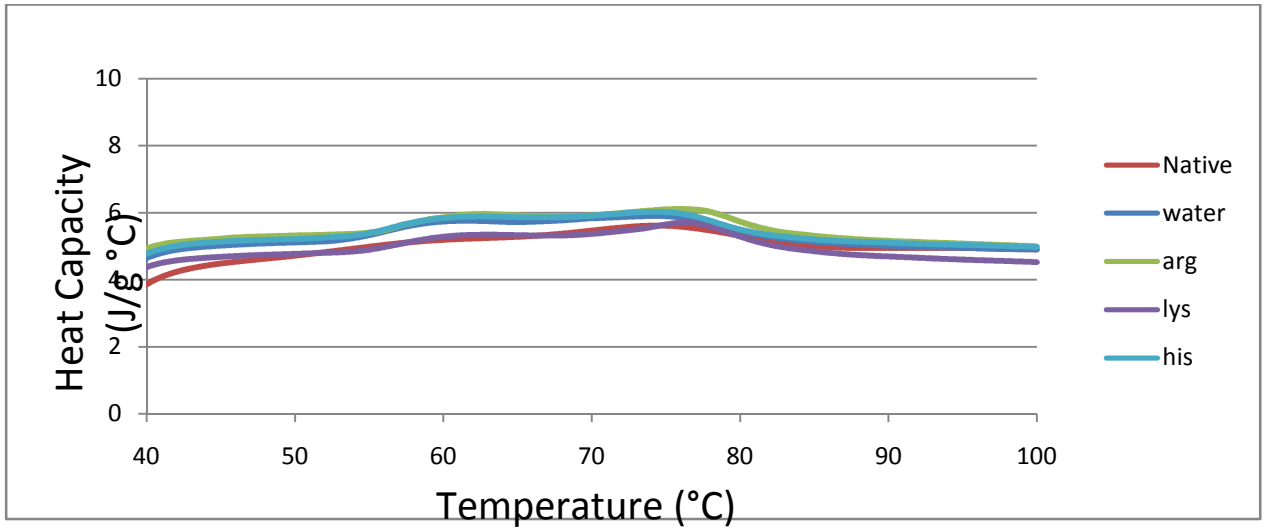

Figure 3.16. DSC thermogram of Oven-dried Evangeline Sweet Potato Starch with Amino Acids alone

Table 3.6. Evangeline Oven-dried DSC Results [1,2,3]

Treatment	pH	Time	Onset temp (°C)	Peak Temp (°C)	Conclusion Temp (°C)	Enthalpy (J/g°C)
Native			66.8±2.06a	75.5±0.48 abc	84.9±2.16a	4.27±1.65a
Control	no	0min	53.2±0.38b	74.6±0.35cde	85.3± 0.1a	15.9±0.07a
Arg	no	0min	54.3±0.39b	76.3±0.18a	86.2±0.69a	14.5±1.67a
Lys	no	0min	54.2±0.38b	76.0±0.28ab	87.8±0.88a	15.7±0.07a
His	no	0min	54.6±0.63b	74.6±0.22bcde	84.7±1.42a	14.6±0.48a
noaa	3	30min	58.1±0.85b	74.2±0.69cde	85.4±0.47a	12.6±1.36a
Arg	3	30min	57.2±1.28b	74.9±0.37abcde	83.3±1.16a	11.6±0.05a
Lys	3	30min	56.7±0.79b	74.7±0.35bcde	86.3±0.53a	14.9±0.18a
His	3	30min	56.3±1.94b	73.6±0.04e	85.0±2.84a	15.8±0.43a
noaa	10	30min	57.5±2.79b	74.2±0.14cde	84.3±1.46a	12.3±2.57a
Arg	10	30min	55.7±0.32b	75.0±0.08abcde	88.4±1.21a	15.4±0.43a
Lys	10	30min	56.3±0.83b	75.2±0.74abcd	86.5±1.39a	14.1±0.86a
His	10	30min	54.9±0.28b	73.8±0.13de	86.7±0.16a	15.3±0.18a

[1]Means in the same column with the same letter are not significantly different at p≥0.05
[2]Control is native starch with water added prior to specify drying method.
[3]Arg is arginine, Lys is lysine and His is histidine

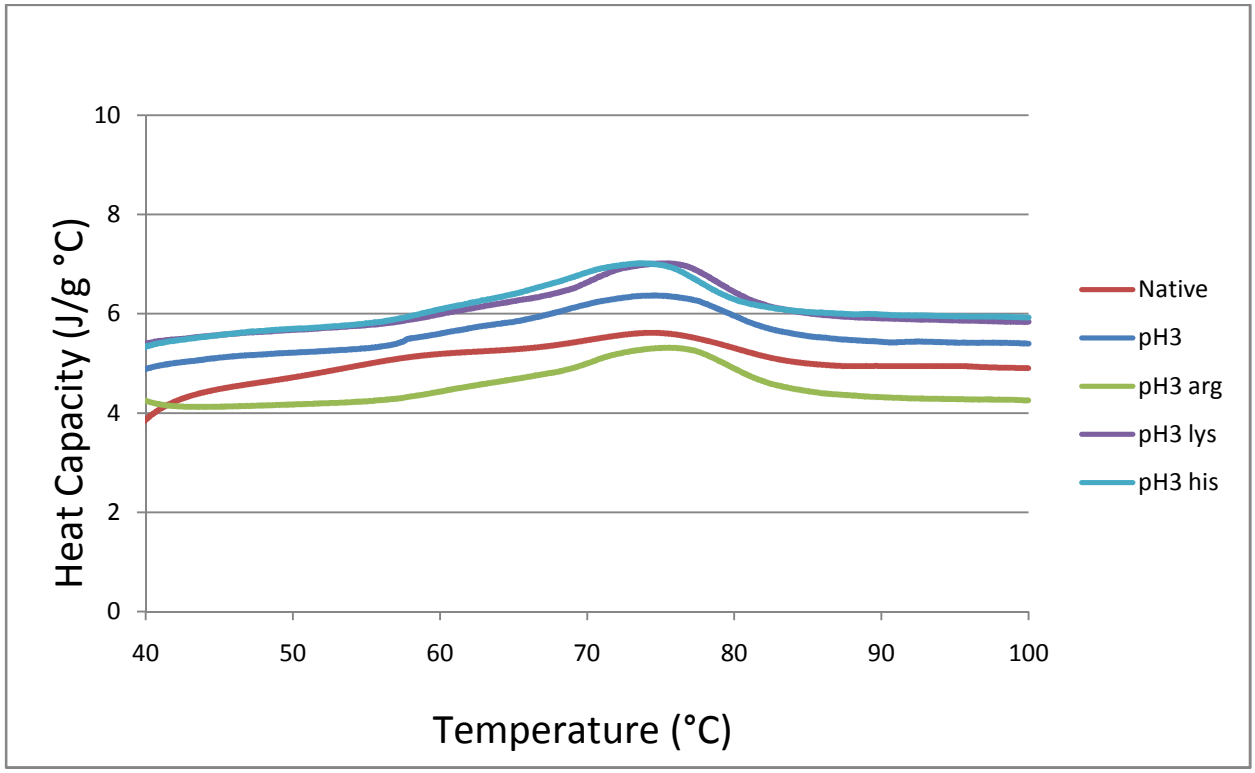

Figure 3.17. DSC thermogram of Oven-dried Evangeline Sweet Potato Starch with Amino Acids at pH 3

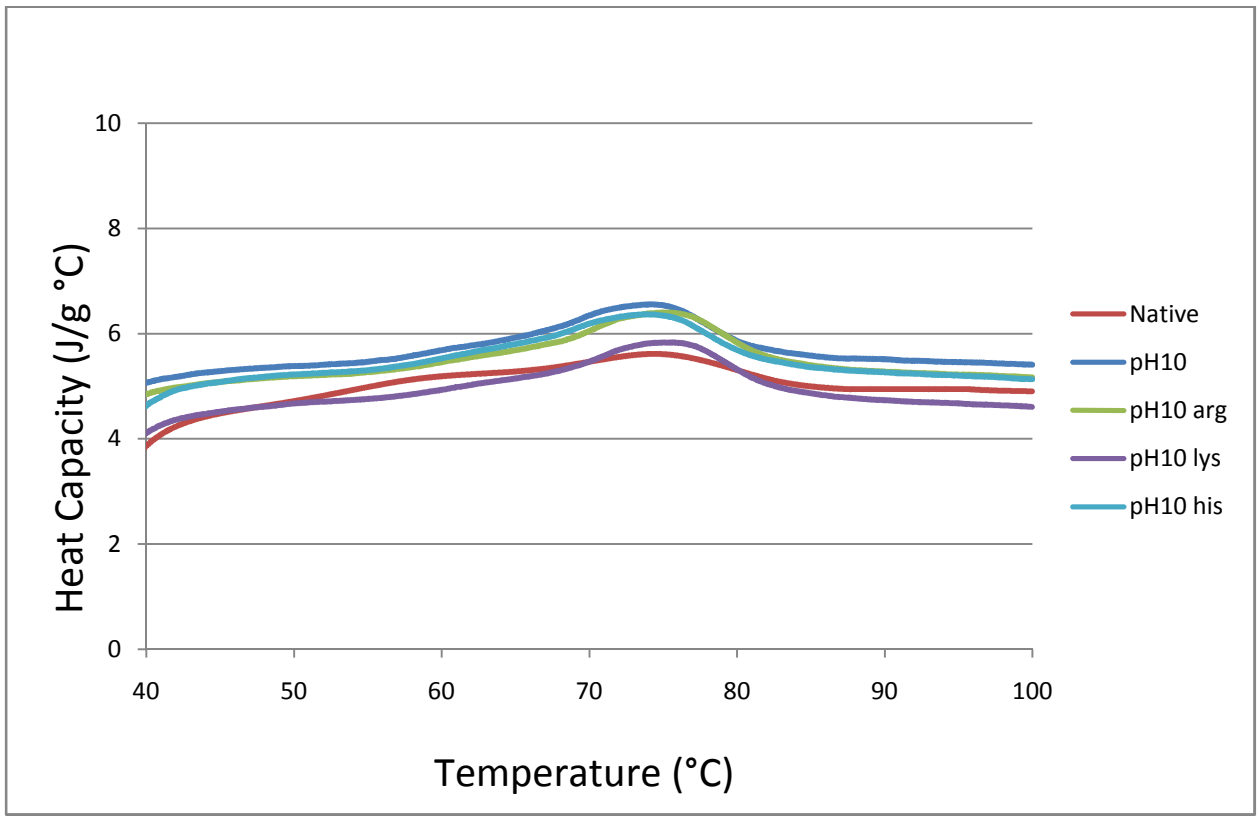

Figure 3.18. DCS thermogram of Oven-dried Evangeline Sweet Potato Starch with Amino Acids at pH 10

3.3.4. Comparison of Gelatinization Characteristics of Beauregard and Evangeline Sweet Potato Starch

Differences were found between the Beauregard and the Evangeline sweet potato starches when compared directly without amino acids or pH additives by t-test. There was a significant difference ($p>0.05$) between the two starches in onset temperature, Beauregard (57.1°C) and Evangeline (66.8°C). The native Beauregard and Evangeline starch did not differ statistically ($p>0.05$) in gelatinization temperature or enthalpy. The onset temperature of the Beauregard starch was about 8°C lower than the Evangeline sweet potato starch although the peak temperatures showed no significant difference. The phosphate content of the two varieties of sweet potato is similar and non-detectable. Kitahara and others (2005) found that the phosphate content of sweet potato starch had a positive relationship with the gelatinization temperature. They also found that with a larger number of phosphate groups a higher gelatinization temperature could be found.

3.4. Conclusion

This study showed that there are noticeable differences between Beauregard and Evangeline starch in terms of their gelatinization characteristics. The Beauregard sweet potato starch had a lower onset temperature than the Evangeline sweet potato starch. Both sweet potato starches had similar temperatures at which the granules gelatinize.

The Beauregard and Evangeline had similar gelatinization temperatures but Beauregard starch required more energy to gelatinize than the Evangeline (Tables 3.3, Table 3.5). For the Beauregard freeze-dried starch, gelatinization temperature were decreased by pH treatments with pH10 decreasing gelatinization temperature to 68.7 °C, as well as histidine at pH10 to 69.7 °C compared to both native and control starch**.** For the Evangeline freeze-dried starch, pH3 alone and histidine treatments at all pH's significantly decreased gelatinization temperature, especially at pH 10 for one hour (73.17 °C) compared to native. Lysine alone caused an increase in gelatinization temperature compared to the control, but was not different from native. For the Beauregard oven-dried starch, the control significantly lowered gelatinization temperature compared to the native. pH 3 and pH 10 were significant in lowering gelatinization temperature of the starch. Lysine and histidine were significant amino acids in decreasing gelatinization temperature compared to native. For the Evangeline oven-dried starch histidine at pH 3 and pH 10 were significant for decreasing gelatinization temperature compared to the native starch. Arginine and lysine were effective additives for increasing gelatinization temperature compared to the control. pH alone did not significantly affect the gelatinization temperature.

CHAPTER 4. EFFECTS OF pH TREATMENT AND AMINO ACID ADDITIVES ON PASTING CHARACTERISTICS OF SWEET POTATO STARCH USING RAPID VISCO ANALYZER (RVA)

4.1. Introduction

Starch pasting takes place by heating the starch with an excess of water until the starch granules begin to swell, this is known as gelatinization. Once the starch is gelatinized and heat is continually applied the starch granules continue to swell causing the starch pasting viscosity to increase (Thomas and Atwell, 1998). Peak viscosity is known as the point at which the starch granules are swollen but the majority of them have not ruptured (Thomas and Atwell, 1998). With continued heating the starch granules begin to rupture and leach out amylose and amylopectin molecules; this point is considered the breakdown of the starch. When heat is no longer applied, the starch begins to cool down and the amylose and amylopectin molecules that leached out of the granule begin to reassociate, known as retrogradation of the starch molecule. (Thomas and Atwell, 1998).

Amino acids have been used to influence pasting characteristics of starches. Lockwood and King (2008) studied the effect of different amino acids of pasting of starch granules and how these starches could be used for various food applications. From their study they found that both lysine, a positively-charged amino acid, and aspartic acid, a negatively charged amino acid, decreased the viscosity of starch paste made from Beauregard sweet potatoes into a thinner pasting starch.

Rheology refers to the study of deformation and flow characteristics of matter in terms of viscosity and shear stress. The study of viscoelastic behaviour oscillatory shear provides valuable information on structural properties of starch changes during heating (Baixauli and others 2008). Viscosity and viscoelasticity of the starch paste can be examined by determining the effect that an oscillating force has on the movement of the material. The change in strain (delta degrees) is

the ratio of the shear stress to the shear strain where G' is the in-phase storage modulus and G" is the out-of-phase similarly-directed loss modulus. (Yoo 2004, Cho and others 2009, Rao 1999)

$$G' = [50/80] \cos \delta$$

$$G'' = [50/80] \sin \delta$$

$$\text{Tan } \delta = G''/G'$$

Sweet potato starch was chosen for this study in order to continue the research on additives that affect the pasting characteristics of sweet potato starch, to change their texture, lower viscosity, and add nutritional value. The objective of this study was 1) to determine the effect different positively charged amino acids would have on sweet potato starch pasting properties, 2) to determine if altering the pH of the amino acids in a solution would affect their binding ability and therefore alter pasting properties of the starch, and 3) to investigate the differences between orange-fleshed Beauregard and Evangeline sweet potatoes by use of RVA.

4.2. Materials and Methods

4.2.1. Materials

Starch was extracted from Beauregard and Evangeline sweet potatoes grown at the LSU AgCenter research station harvested in September of 2008. In this study positively charged amino acids with pH treatment at pH 3 and 10 were used. The amino acids were purchased from Sigma Chemical Company (St. Louis, Missouri). Amino acids used included positively charged aginine, lysine and histidine. These amino acids were chosen based upon past research (Liang 2001, An 2005 and Lockwood 2008).

4.2.2. Sweet Potato Starch Extraction

See section 3.2.2. for Sweet Potato Starch Extraction procedure

4.2.3. Starch Treatment

See section 3.2.3 for Starch Treatment procedure

4.2.4. Proximate Analysis

See section 3.2.5. for Proximate Analysis procedure

4.2.5. Rapid Visco Analyzer Analysis

A rapid Visco Analyzer 3D (Newport Scientific, Warriewood, Australia) was used to determine pasting pasting properties. Samples were made for the RVA on a 7% dry weight starch basis, based on preliminary study, plus amino acid additives on a 6% dry weight basis of the starch (Liang, 2001). Water was added to a total of 28g (starch, amino acid, and water). The following equations were used to determine the amount of starch:

(7/100) = (x/28), x= 1.97g dry starch

100g- moisture content = dry starch weight

1.96/ dry starch weight = grams of wet starch

Grams of dry starch x 6% = grams of amino acid

28- (starch + amino acid) = grams of water

Table 4.1. RVA Procedure

Process	Time (min)
Hold 50°C	1:00
Ramp 12°/min from 50 -90°C	4:45
Hold at 95°C	7:15
Ramp 12°/min from 95-50°C	11:00
Hold 50°C	13:00

The actual moisture content of the starch was determined by using a moisture analyzer. The combined water, starch and amino acids were mixed several times to ensure proper combination of the water and starch. The sample was then placed into the RVA, which was programmed using the thirteen-minute method of Shin and others (2004), which is specific to sweet potatoes. The RVA procedure started by holding the starch for one minute at 50°C then the mixture was

heated to 95°C at a ramp of 12°C/minute, the starch was then held at 95°C for 2.5 min, and was cooled to 50°C at 12°C/ minute. Throughout the process, the rotating speed of the RVA was kept constant at 160 rpm. The following table illustrates the procedure used:

The measurement for time, temperature, and viscosity were collected and analyzed. The RVA measured several points including: peak viscosity (PV), minimum viscosity (MV), final viscosity (FV), time to peak (P-time), and pasting temperature (PT). Total setback (TSB) and breakdown (BD) were calculated using the equations: FV-MV = TSB and PV-MV = BD. All samples were analyzed in duplicated.

4.2.6. Rheology Analysis

Rheology properties, G' and G" moduli, of native starch samples were measured using an AR 2000 EX Rheometer, (TA Instruments, New Castle, DE) fitted with plate geometry acrylic plates with a 40-mm diameter having a 200 µm gap between plates. Samples were prepared prior to analysis on a 7 % starch to water solution for both Beauregard and Evangeline sweet potato starch. An frequency sweep test was used to study rheological properties of Beauregard and Evangeline sweet potato starch. Frequency was set at 1Hz and 3 % strain was used. The starch was heated from 50 °C to 95 °C at 12 °C per minute.

4.2.7. Statistical Analysis

SAS (Statistical Analysis System) software (version 9.1) was used to analyze the RVA data. Standard deviation, ANOVA (Analysis of Variance), and Tukey's Studentized Range (HSD) were used to examine the effects of the amino acid additives on the pasting properties of Beauregard and Evangeline sweet potato starches, on a $p \leq 0.05$ level. The abbreviation used were Beau for Beauregard sweet potato, Evan for Evangeline sweet potato, NOAA for no amino acid additives, Arg for arginine, Lys for lysine, His for histidine, PV for peak viscosity, MV for minimum viscosity, BD for breakdown, FV for final viscosity, TSB for total setback, Tp for time to peak, and PT for pasting temperature.

4.3. Results and Discussion

4.3.1. Comparison of Pasting Characteristics of Beauregard and Evangeline Sweet Potato Starch

When the pasting parameters between native Beauregard and Evangeline sweet potato starches were directly compared significant differences were found for PV, BD and PT (Figure 4.1). The two starches had similar MV and FV, as well as TSB and Tp values. These similar pasting characteristics indicated that the two starches could have similar cooking times and similar possibility of retrogradation. The difference between the Beauregard and Evangeline starches indicated that the Beauregard starch would be less stable to cooking with a lower cooking temperature and make a thicker paste. The native Evangeline starch would have a thinner paste and greater stability to shear during cooking.

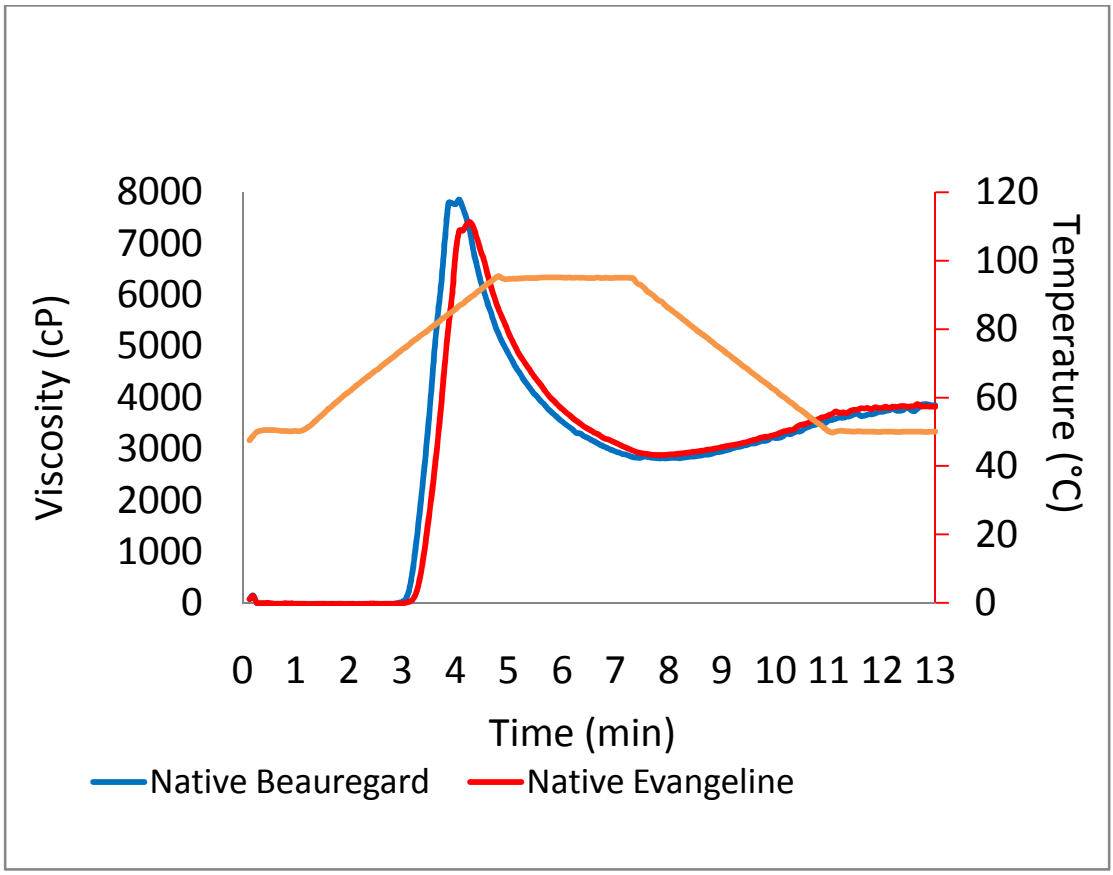

Figure 4.1. Pasting Curve of Native Beauregard and Evangeline Sweet Potato Starch

4.3.2. Effect of Amino Acid Additives, pH Treatment and Time on the Pasting Characteristics of Beauregard Sweet Potatoes

For the freeze-dried Beauregard sweet potato starch, all amino acids decreased PV except for arginine and lysine at pH3 compared to native and control (Table 4.2 and Figures 4.2 to 4.6). Lockwood and King (2008) found that lysine decreased PV compared to native orange-fleshed sweet potato starch. The treatment at pH3 alone for 30 min and histidine at pH3 for 30 min decreased PV, pH10 alone for 30 min increased PV while the addition of amino acids at pH10 for 30 min decreased PV. PV decreased with treatment at pH10 alone for one hour and further decreased with the addition of amino acids. (Table 4.2 and Figure 4.6) Thirathumthavorn and Charoenrein (2005) found that pasting viscosities decreased as acid hydrolysis increased.

BD, calculated by subtracting the MV from the PV, decreased with the addition of histidine alone, pH 3 for 30 min, and pH 3 for one hour, compared to control and native starch. Lockwood and King (2008) found that charged amino acids decreased PV, MV, FV and BD compared to native orange-fleshed sweet potato starch. FV decreased with the addition of lysine at pH10 for 30 min; the other treatments did not significantly decrease the FV. Liang and King (2003) found that charged amino acids decreased MV and FV in rice starch. TSB, calculated by subtracting the MV from the FV and Tp, did not change compared to native and control. PT was significantly changed by various starch treatments (Table 4.2). Arginine and lysine alone and with pH treatments increased PT compared to control and native starch. These findings agree with the results obtained by Lockwood and King (2008) who found that charged amino acids increased PT compared to native orange-fleshed sweet potato starch. Histidine alone and with pH treatments decreased PT compared to native starch. pH treatments alone decreased PT compared to native starch, pH treatments for 30 min alone decreased PT compared to control and native starch to 70.8 °C for 30 min at both pH 3 and 10. (Table 4.2)

Table 4.2. Pasting Properties Beauregard Freeze-dried samples [1,2,3,4,5]

AA	pH	Time	PV	MV	BD	FV	TSB	Tp	PT
Native			7932± 103b	2790± 39ab	5142± 142ab	3901± 71a	1111± 110ab	3.97± 0.14def	72.4± 0.0c
control	no	0min	7823± 9.19b	2893 ± 22ab	4930± 31abcd	3939± 9.9a	1046± 32ab	4.2± 0.0abc	71.6± 0.07d
Arg	no	0min	6287± 12hi	1642± 74b	4644± 62abcde	3292± 74ab	1650± 0.0a	4.27± 0.0ab	73.9± 0.04a
Lys	no	0min	6190± 158i	2033± 37b	4157± 120def	3328± 55ab	1294± 16ab	4.3± 0.042a	74.0± 0.04a
His	no	0min	6581± 57fghi	2583± 9.192ab	3998± 66ef	3465± 23ab	882± 32ab	4.07± 0.0cde	71.6± 0.07d
noaa	3	30min	6805± 54efg	2588± 14ab	4217± 69defghi	3509± 51ab	920± 36ab	3.9± 0.04ef	70.8± 0.0f
Arg	3	30min	7497± 12bc	2394± 7.1ab	5103± 5abc	3760± 23ab	1366± 29ab	4.0± 0.0def	73.2± 0.04b
Lys	3	30min	7277± 190bc	2411± 39ab	4865± 152abcde	3620± 18ab	1208± 57ab	4.04± 0.05cdef	73.1± 0.10b
His	3	30min	7025± 43def	2647± 9ab	4378± 33bcde	3479± 18ab	832± 28b	4.0± 0.0def	71.6± 0.07d
noaa	10	30min	8325± 265a	2936± 75ab	5388± 190a	4003± 91a	1067± 15ab	3.97± 0.05def	70.8± 0.04ef
Arg	10	30min	6912± 40efghi	2231± 49ab	4681± 9abcde	3435± 24ab	1204± 25ab	4.04± 0.05cdef	73.2± 0.0b
Lys	10	30min	6925± 149defg	3423± 1540a	4038± 931def	3455± 106b	600± 842b	4.07± 0.0cde	73.3± 0.14b
His	10	30min	6987± 100def	2641± 39ab	4345± 139bcde	3497± 8.5ab	855± 47b	4.04± 0.05cdef	71.5 ± 0.0de
noaa	3	1hour	5376± 30j	2041± 7.1b	3335± 37f	2850± 28ab	809± 21b	3.87± 0.0ef	71.6 ± 0.0d
Arg	3	1hour	6521± 255ghi	2084± 118ab	4437± 137bcde	3392± 2.8ab	1307± 120ab	4.07± 0.0cde	73.7± 0.53ab
Lys	3	1hour	6776± 42fg	2376± 1.41ab	4400± 41bcde	3420± 115ab	1044± 114b	4.07± 0.0cde	73.2± 0.04b
His	3	1hour	6806± 20efg	2636± 31ab	4170± 52def	3476± 1.4ab	839± 30b	4.00± 0.0def	71.6 ± 0.07d
noaa	10	1hour	7232± 6cde	2763± 33ab	4469± 26bcde	3602± 26.2ab	839± 59b	4.00± 0.0def	71.6± 0.07d
Arg	10	1hour	6525± 96ghi	2073± 22ab	4452± 74bcde	3330± 16.3ab	1257± 39ab	4.1± 0.04bcd	73.7± 0.49ab
Lys	10	1hour	6672± 24fgh	2266± 2.12ab	4405± 22bcde	3292± 27.6ab	1026± 30ab	4.04± 0.05cdef	73.2± 0.03b
His	10	1hour	6672± 8fgh	2589± 16ab	4082± 7.8def	3467± 2.8ab	877± 13ab	4.04± 0.04cdef	71.6± 0.07d

[1] Means in the same column with the same letter are not significantly different at p≥0.05

[2]Control is starch with water added prior specified drying method.

[3]Arg is arginine, Lys is lysine and His is histidine

"Table Continued"

[4]AA is amino acid, PV is peak viscosity, MV is minimum viscosity, BD is breakdown, FV is final viscosity, TSB is total setback, Tp is time to peak and PT is pasting temperature.
[5]Units = viscosity (cP); temperature (ºC); time (min.)

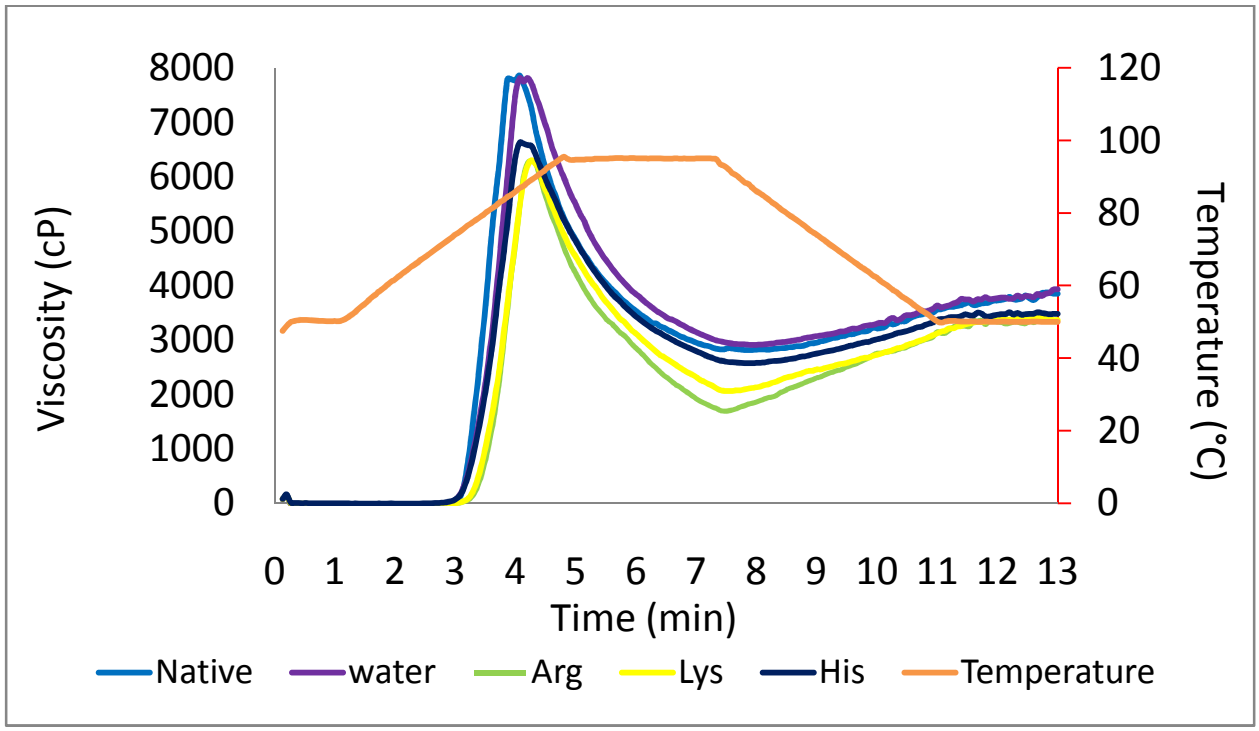

Figure 4.2. Pasting curve of Freeze-dried Beauregard Starch with Amino Acids

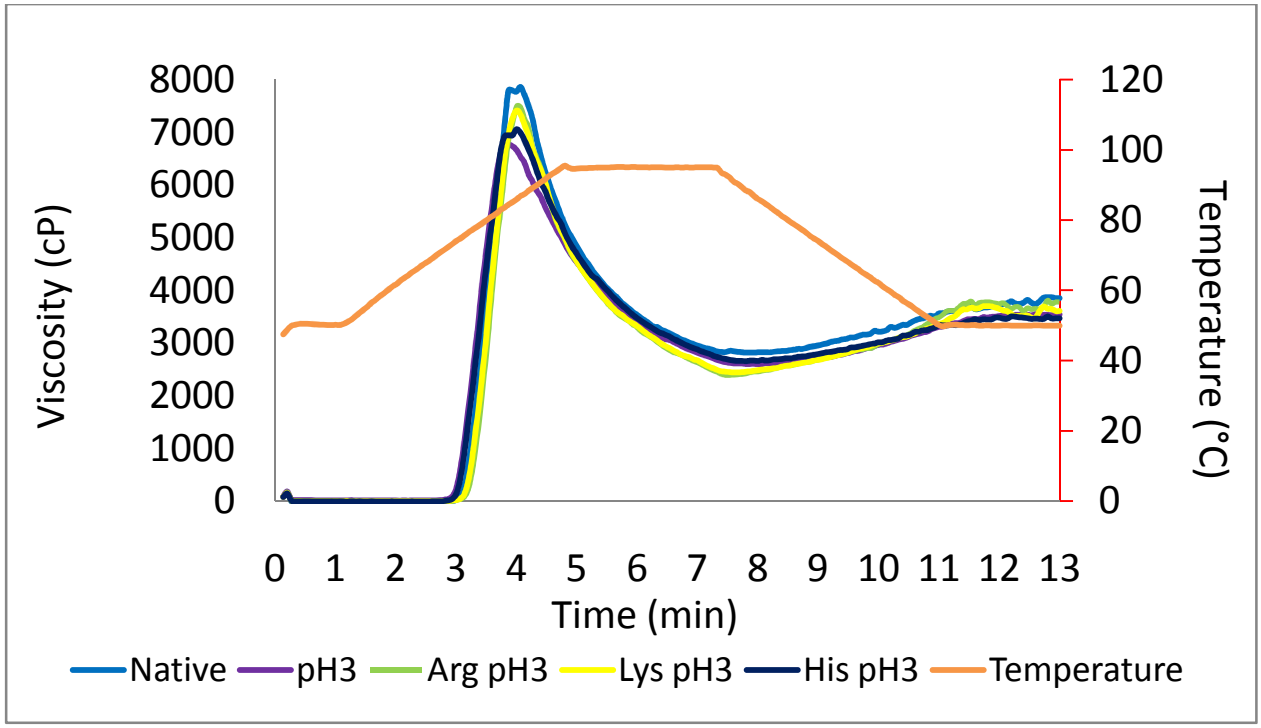

Figure 4.3. Pasting curve of Freeze-dried Beauregard Starch at pH3 for 30 min

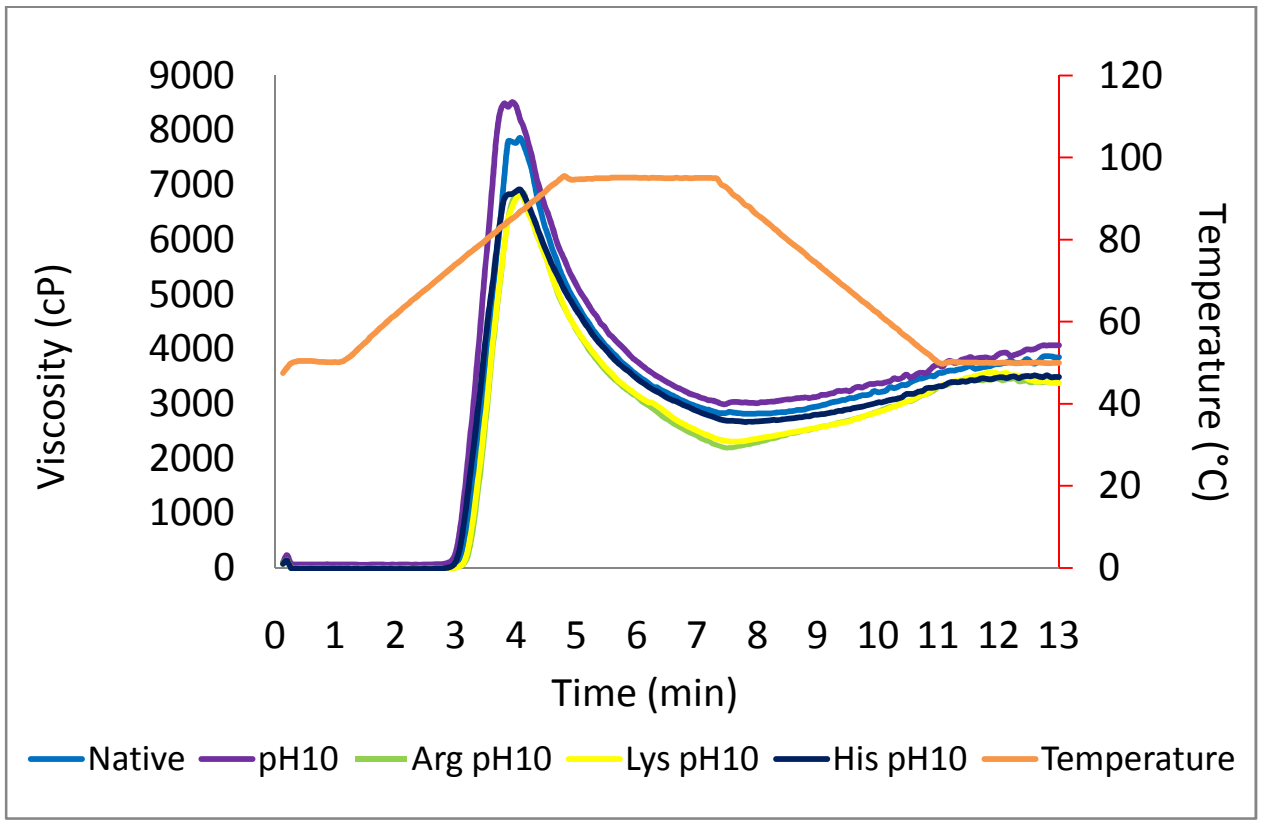

Figure 4.4. Pasting curve of Freeze-dried Beauregard Starch at pH10 for 30 min

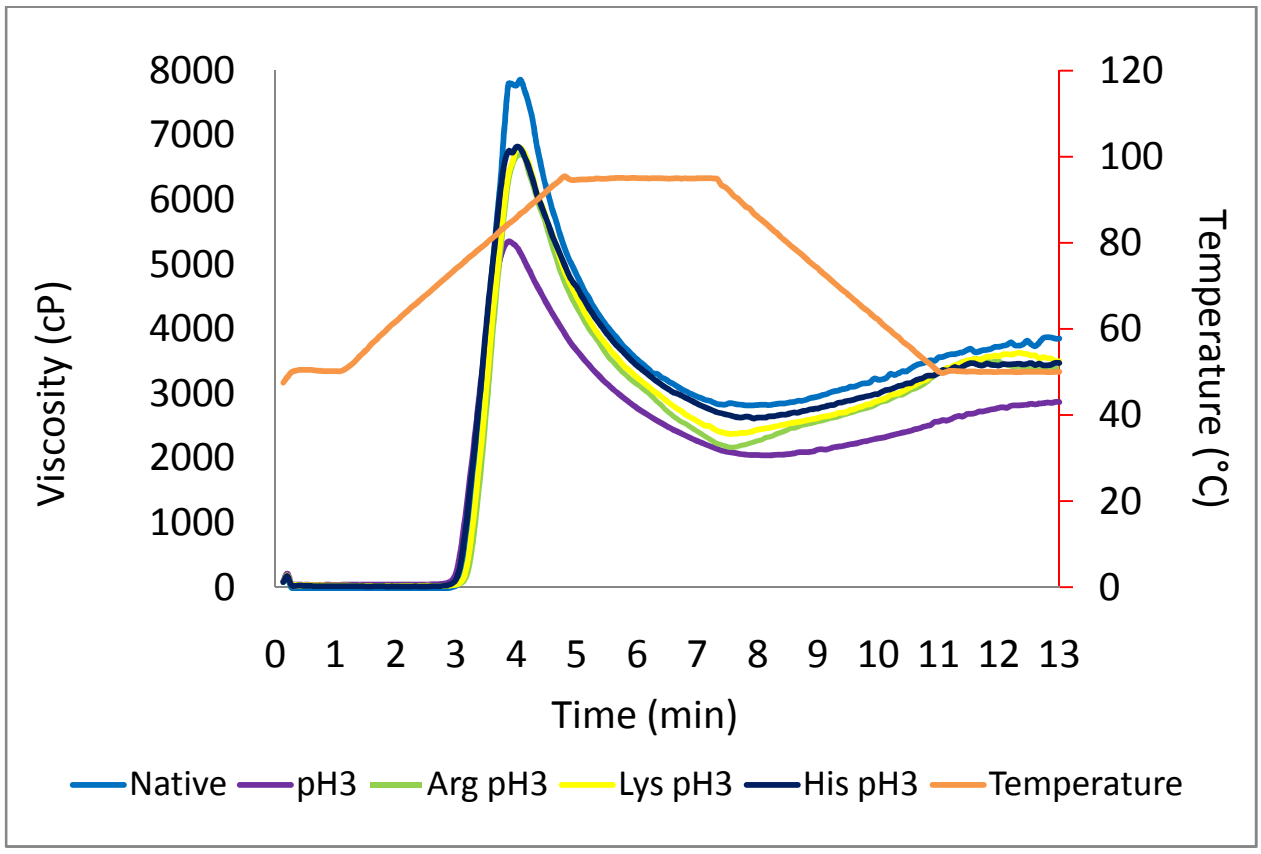

Figure 4.5. Pasting curve of Freeze-dried Beauregard Starch at pH3 for 1 hour

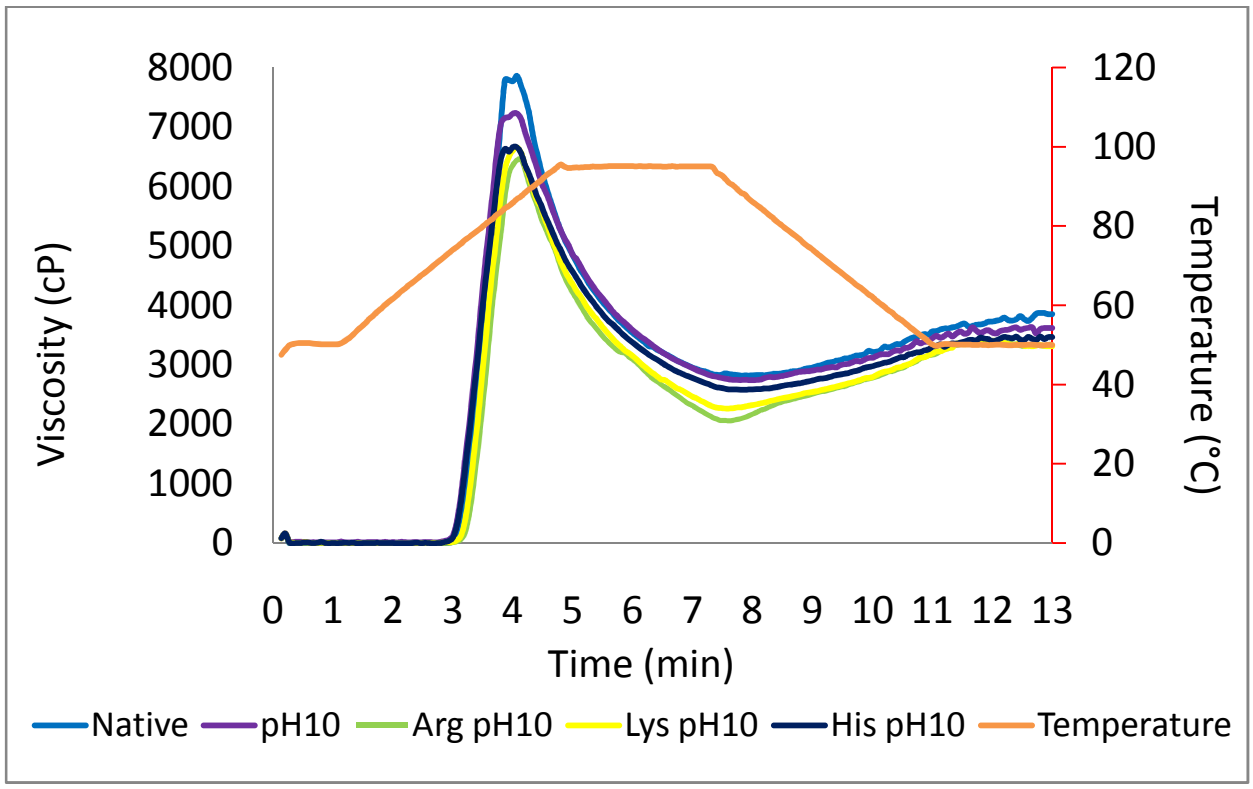

Figure 4.6. Pasting curve of Freeze-dried Beauregard Starch at pH10 for 1 hour

For the oven-dried Beauregard starch treated with amino acids and pH treatments decreased the PV, MV, BD, FV and TSB compared to control and native starch, except for lysine alone for BD; arginine alone for FV; histidine alone, pH 3 and pH 10 alone, and histidine pH 10 for TSB (Table 4.3 and Figure 4.7 to 4.9). Liang and King (2003) found that charge amino acids decreased cooking stability of the starch but arginine and lysine increased retrogradation tendency. Lockwood and King (2008) found that lysine decreased BD in orange-fleshed Beauregard sweet potato starch. The largest decrease in PV at 2923 cP was found for lysine with pH 10, showing that the starch was modified into a thinner paste (Figure 4.9). The largest decrease in MV at 1453 cP was found with lysine pH 10, producing a starch that is easier to cook. The largest decrease in BD at 1461 cP was found with starch treated with lysine pH 3, this signifies that the paste will be more stable to shear during cooking. Chung and others (2003) studied the effect of acid hydrolysis on pasting properties of high amylose corn starch and found that acid hydrolysis decreased paste viscosity which may be due to the acid decreasing the chain

length. The largest decrease in FV was found with lysine at pH10 with 2113cP (Table 4.3). Our results were similar to Bao and Corke (2002) who found that viscosity characteristics decreased under stronger alkaline (pH 11.5) or acidic (pH 2.5) conditions. Arginine and lysine without pH adjustment increased TSB to 1638 cP and 1381 cP respectively. An increase in TSB indicates a higher possibility for retrogradation. With pH adjustment, arginine and lysine decreased TSB compared to control and native starch. Pasting times did not change compared to control. PT increased in both pH 3 and 10 treatments with and without amino acids compared to the control and native starch (Table 4.3), demonstrating that the starch begins to swell at a higher temperature. Lockwood and King (2008) found that orange-fleshed sweet potato starch with added lysine showed that the starch granules begin to swell at higher temperature than the native starch.

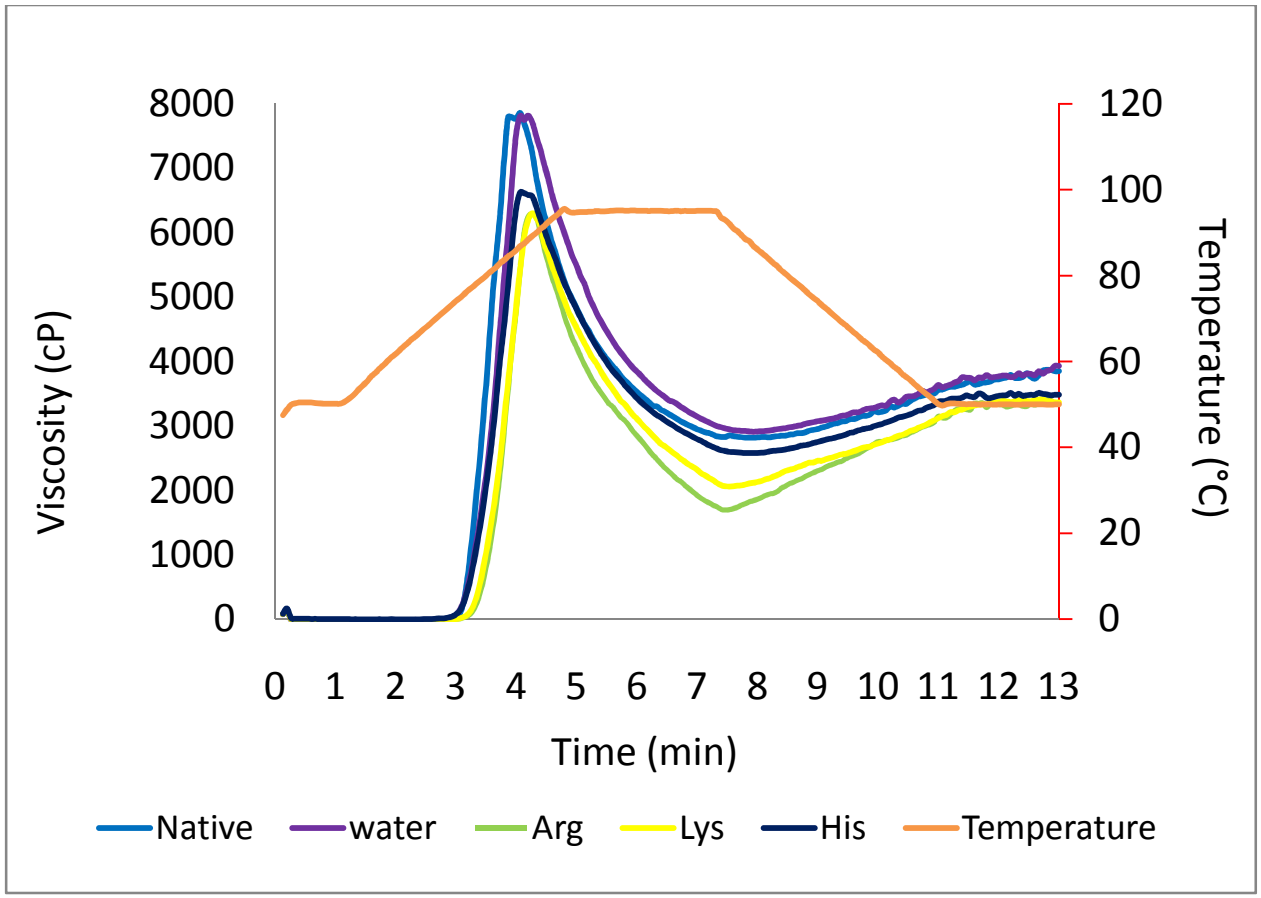

Figure 4.7. Pasting curve of Oven-dried Beauregard Starch with Amino Acids

Table 4.3. Pasting Properties Beauregard Oven-dried samples [1,2,3,4,5]

AA	pH	Time	PV	MV	BD	FV	TSB	Tp	PT
Native			7932± 102a	2790± 39.6a	5142± 142a	3901± 70.7a	1111± 110c	3.97± 0.14b	72.4± 0.0d
control	no	0min	5872± 72.8b	2340± 33.2b	3532± 39.6c	3264± 23.3b	924± 9.9d	4.2± 0.0a	69.2± 0.07f
Arg	no	0min	5400± 41.7c	1543± 19.1fg	3857± 60.8b	3182± 8.49b	1638± 27.6a	4.27± 0.0a	70.78± 0.04e
Lys	no	0min	5262± 22.6c	1599± 63.6f	3663± 86.3bc	2980± 34.7c	1381± 29b	4.27± 0.0a	70.33± 0.6ef
His	no	0min	5015± 69.3d	2069± 54.5c	2945± 14.9d	2920± 37.5c	851± 16.9def	4.2± 0.0a	69.48± 0.6ef
noaa	3	30min	3790± 12f	1857± 15.6de	1933± 27.6 f	2687± 22.6d	830± 7.07defg	4.13± 0.00ab	73.25± 0.0bcd
Arg	3	30min	2997± 0.71 h	1455± 40.3g	1542± 39.6gh	2149± 58.7f	694± 18.4gh	4.24± 0.05a	74.85± 0.00a
Lys	3	30min	2941± 2.83h	1480± 5.7fg	1461± 8.5h	2137± 12f	657± 6.4h	4.27± 0.00a	74.40± 0.49abc
His	3	30min	3235± 39.6g	1732± 14.1e	1503± 25.5h	2485± 65.8e	753± 51.6efgh	4.20± 0.00a	73.63± 0.6abcd
noaa	10	30min	4158± 19.1 e	1946± 7.07cd	2212± 26.2 e	2823± 4.95cd	877± 12de	4.13± 0.00ab	73.55± 0.6abcd
Arg	10	30min	3258± 24g	1524± 41.7fg	1733± 17.7fg	2249± 72.83f	725± 31fgh	4.24± 0.05a	74.78± 0.04ab
Lys	10	30min	2923± 11.3h	1453± 5.7g	1470± 16.9h	2113± 0.71f	660± 6.4h	4.27± 0.00 a	74.43± 0.53abc
His	10	30min	3641±5 5.2f	1880± 19.8d	1761± 35.4 f	2731± 22.6d	851± 2.8def	4.20± 0.00a	73.20± 0.0cd

[1] Means in the same column with the same letter are not significantly different at p≥0.05

[2]Control is starch with water added prior specified drying method.

[3]Arg is arginine, Lys is lysine and His is histidine

[4]AA is amino acid, PV is peak viscosity, MV is minimum viscosity, BD is breakdown, FV is final viscosity, TSB is total setback, Tp is time to peak and PT is pasting temperature.

[5]Units = viscosity (cP); temperature (ºC); time (min.)

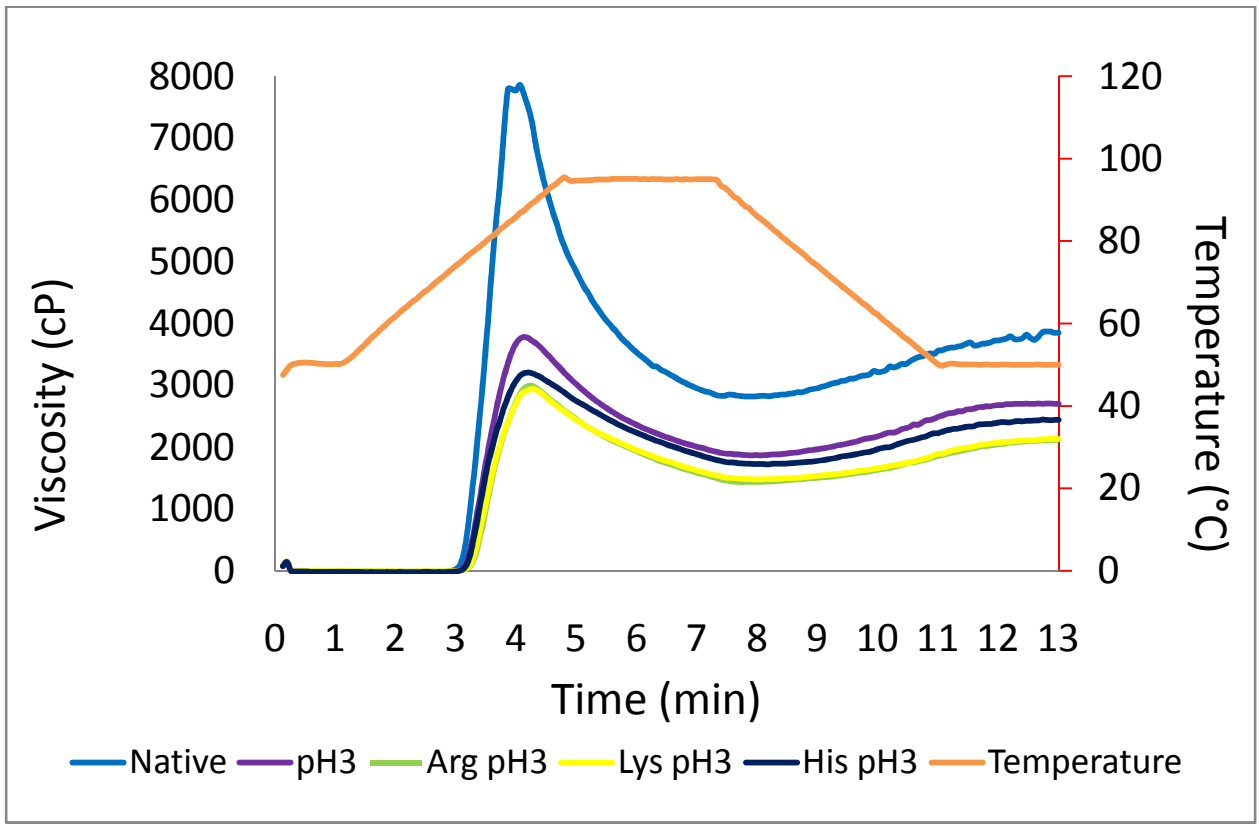

Figure 4.8. Pasting curve of Oven-dried Beauregard Starch at pH 3

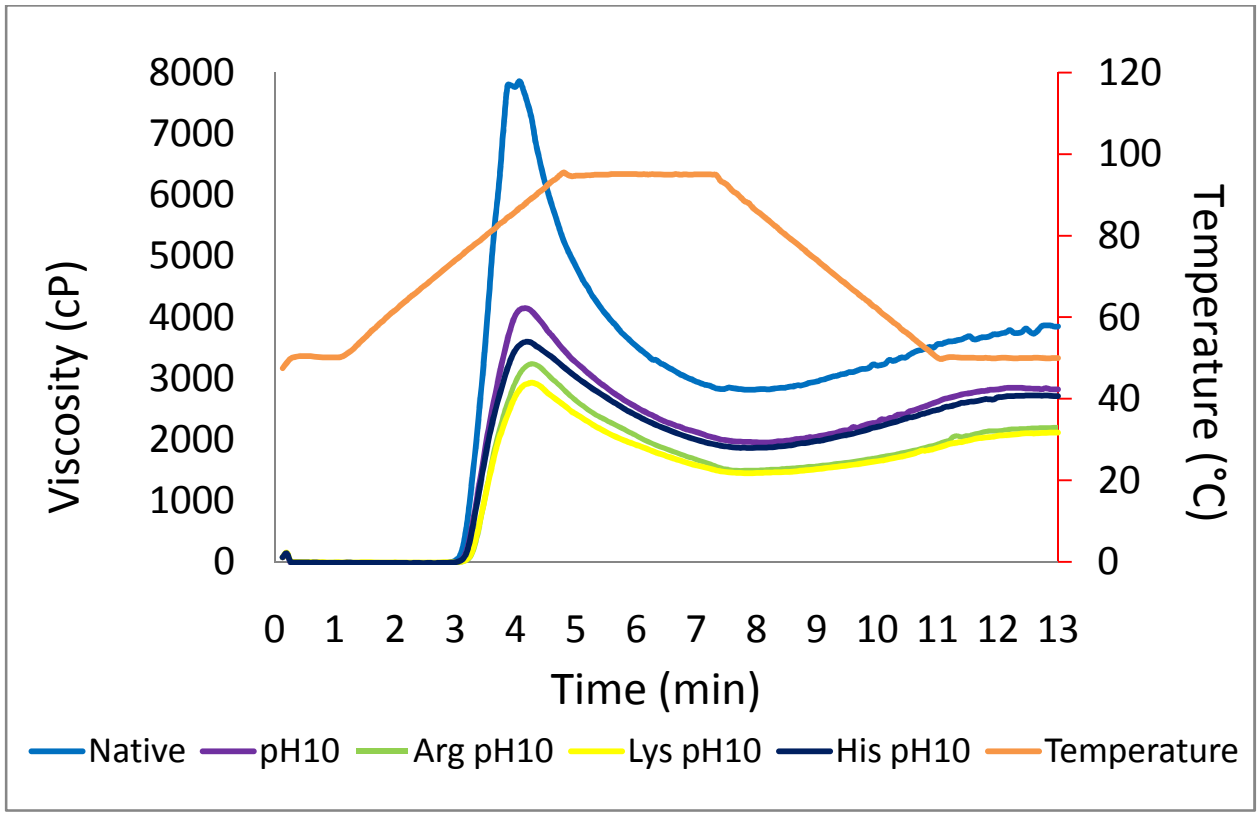

Figure 4.9. Pasting curve of Oven-dried Beauregard Starch at pH 10

4.3.3. Effect of Amino Acid Additives, pH Treatment and Time on the Pasting Characteristics of Evangeline Sweet Potatoes

For the freeze-dried Evangeline sweet potato starch arginine, lysine and histidine alone and lysine at pH 10 for 30 min significantly decreased PV compared to control and native starch (Table 4.4 and Figures 4.11 to 4.14). MV was decreased the most with the addition of amino acids alone.

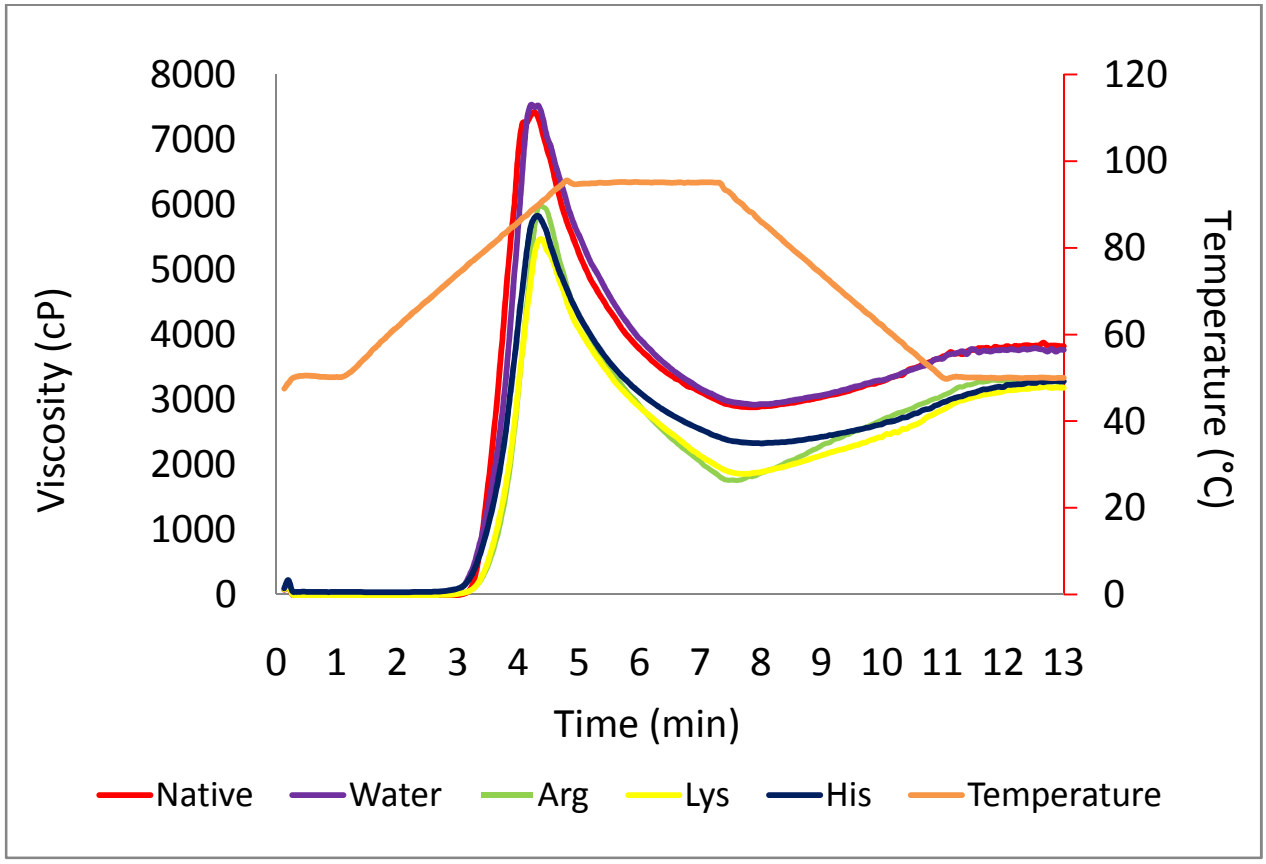

Figure 4.10. Pasting curve of Freeze-dried Evangeline Starch with Amino Acids

There was a decrease found with arginine at pH 3 and pH 10 for 30 min and one hour. MV also decreased with the addition of lysine at pH 10 for 30 min, pH 3 for one hour and lysine at pH10 for one hour. BD decreased the most with lysine and histidine alone and also decreased with the addition of lysine pH10 for 30 min (Table 4.4). FV decreased significantly with the addition of arginine and lysine alone compared to control and native starch (Figure 4.10). These findings agree with the results obtained by Lockwood and King (2008) who found that charged amino

acids decreased PV, MV, FV and BD compared to native orange-fleshed sweet potato starch. TSB increased with the addition of arginine and lysine alone. Time to peak was not significantly changed. Peak temperature decreased with pH 3 at 30 min and one hour compared to native starch. PT increased with the addition of arginine at pH 3 and pH 10 for 30 min, lysine pH 3 and pH 10 for 30 min and one hour, and arginine pH 10 for 30 min and one hour (Table 4.4). These findings agree with the results obtained by Lockwood and King (2008) found that charged amino acids increased PT compared to native orange-fleshed sweet potato starch. Bao and Corke (2002) studied the effects of pH on pasting properties of native and γ-irradiated rice starches and found that with a combination of irradiation and acid treatments starch viscosities decreased.

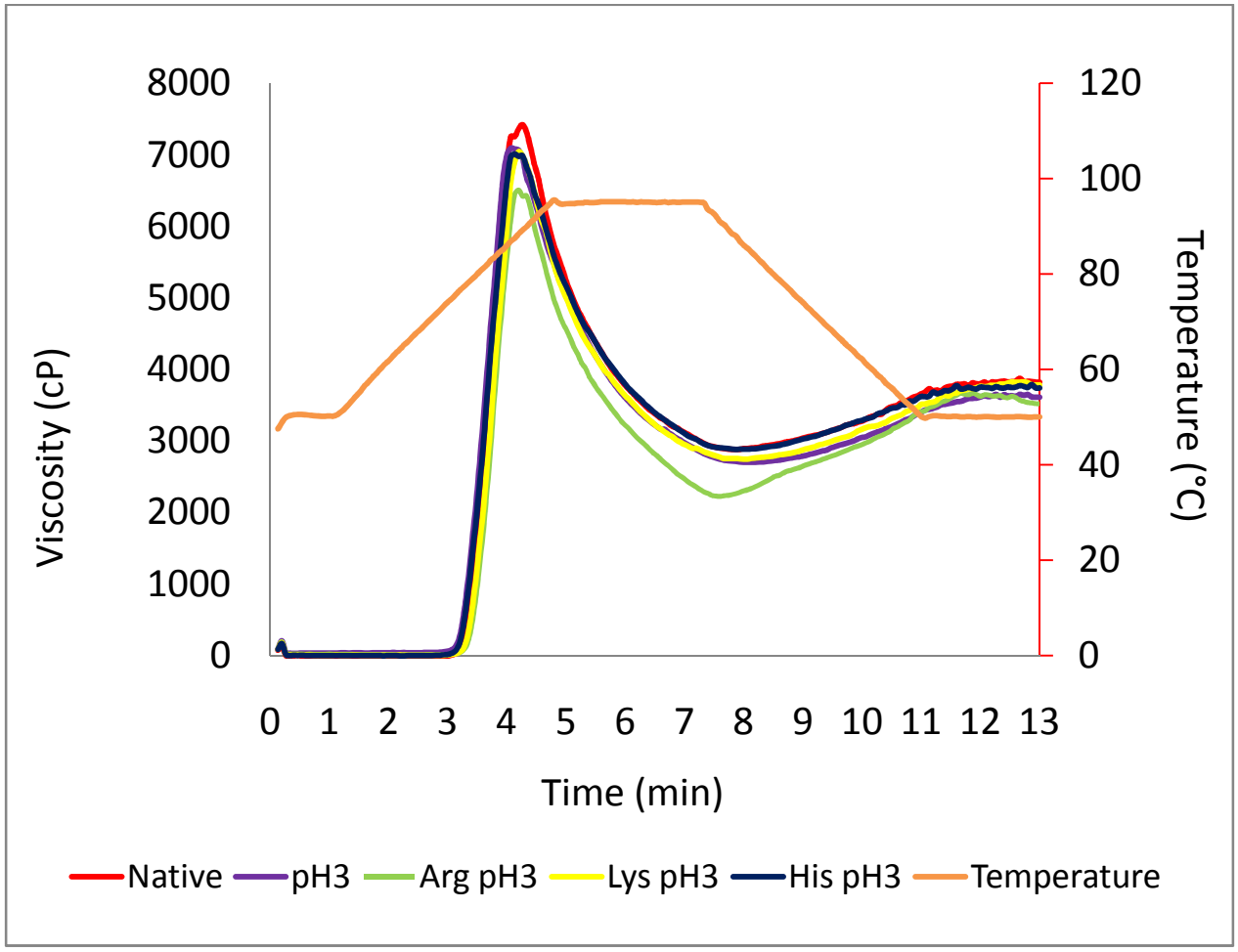

Figure 4.11. Pasting curve of Freeze-dried Evangeline Starch at pH 3 for 30 min

Table 4.4. Pasting Properties Evangeline Freeze-dried samples[1,2,3,4,5]

AA	pH	Time	PV	MV	BD	FV	TSB	Tp	PT
Native			7458± 58ab	2904± 33.9abc	4554.0± 24ab	3820± 0.71a	916± 34.7cde	4.24± 0.05a	74.03± 0.04b
control	no	0min	7530± 9.2ab	2926± 9.9ab	4604.5± 0.71ab	3767± 7.78ab	841± 2.12e	4.2± 0.0a	71.55± 0.07d
Arg	no	0min	5748± 323de	1652± 144g	4096.0± 178bcde	3267± 33.9cd	1615± 178a	4.37± 0.05a	74.00± 0.0b
Lys	no	0min	5402± 77e	1861± 6.36g	3541± 83e	3166± 19.8d	1304± 26.2ab	4.4± 0.0a	73.95± 0.0b
His	no	0min	5989± 236de	2369± 60.8f	3620± 175de	3369± 126bcd	1000± 65.8bcde	4.33± 0.0a	71.58± 0.11d
noaa	3	30min	7032± 78.5abc	2696± 4.95abcd	4336± 74abc	3608± 5.66abc	92± 0.71de	4.07± 0.0a	73.15± 0.0c
Arg	3	30min	6855± 493abcd	2363± 200f	4492± 292ab	3602± 82.7abc	1239± 82.7bc	4.2± 0.0a	74.78± 0.04a
Lys	3	30min	7024± 22.6abc	2722± 32.53abcd	4302± 9.9abc	3795± 24.8a	1073± 57.3bcde	4.2± 0.0a	74.88± 0.04a
His	3	30min	7127± 166abc	2900± 28.28abc	4227± 137abcd	3758± 26.9ab	858± 1.4e	4.1± 0.04a	73.25± 0.0c
noaa	10	30min	7781± 10.6a	2994± 29a	4787± 39.6a	3817± 32.5a	822± 3.5e	4.14± 0.09a	73.2± 0.07c
Arg	10	30min	7050± 41.7abc	2500± 67.9def	4550± 26.2ab	3618± 100abc	1118± 168bcde	4.24± 0.05a	74.78± 0.04a
Lys	10	30min	6298± 821cde	2500± 202def	3798± 619cde	3534± 351abcd	1034± 149bcde	4.14± 0.09a	74.83± 0.04a
His	10	30min	7135± 73.5abc	2834± 28.3abc	4300± 45.5abc	3714± 6.36ab	879± 34.7e	4.1± 0.04a	73.3± 0.0c
noaa	3	1hour	6857± 4.95abcd	2604± 9.9cdef	4253± 4.95abcd	3562± 24.8abcd	958± 14.9cde	4.39± 0.45a	73.2± 0.0c
Arg	3	1hour	6975± 6.36abc	2401± 42.4ef	4546± 86ab	3503± 86.3abcd	1101± 128bcde	4.2± 0.0a	74.13± 0.6a
Lys	3	1hour	6946± 303abc	2632± 5.7bcdef	4314± 24abc	3753± 13.4ab	1121± 19bcde	4.2± 0.0a	74.83± 0.04a
His	3	1hour	7137± 57abc	2898± 8.5abc	4239± 5.1abcd	3769± 16.3ab	871± 24.8e	4.2± 0.0a	73.18± 0.04c
noaa	10	1hour	7634± 14.8ab	2998± 21a	4636± 5.7ab	3867± 11.3a	868± 31.8e	4.2± 0.0a	73.18± 0.04c
Arg	10	1hour	6986± 106abc	2411± 63.6ef	4575± 42.4ab	36636± 168abc	1225± 105bcd	4.2± 0.0a	74.83± 0.04a
Lys	10	1hour	6802± 30.4bcd	2610± 6.36cdef	4192± 36.7abcde	3715± 74ab	1104± 80bcde	4.2± 0.0a	74.83± 0.04a
His	10	1hour	7121± 42.5abc	2878± 15.6abc	4243± 34abcd	3726± 4.24ab	848± 19.8e	4.2± 0.0a	73.23± 0.11c

[1] Means in the same column with the same letter are not significantly different at $p \geq 0.05$

[2]Control is starch with water added prior specified drying method.

[3]Arg is arginine, Lys is lysine and His is histidine

[4]AA is amino acid, PV is peak viscosity, MV is minimum viscosity, BD is breakdown, FV is final viscosity, TSB is total setback, Tp is time to peak and PT is pasting temperature.

[5]Units = viscosity (cP); temperature (ºC); time (min.)

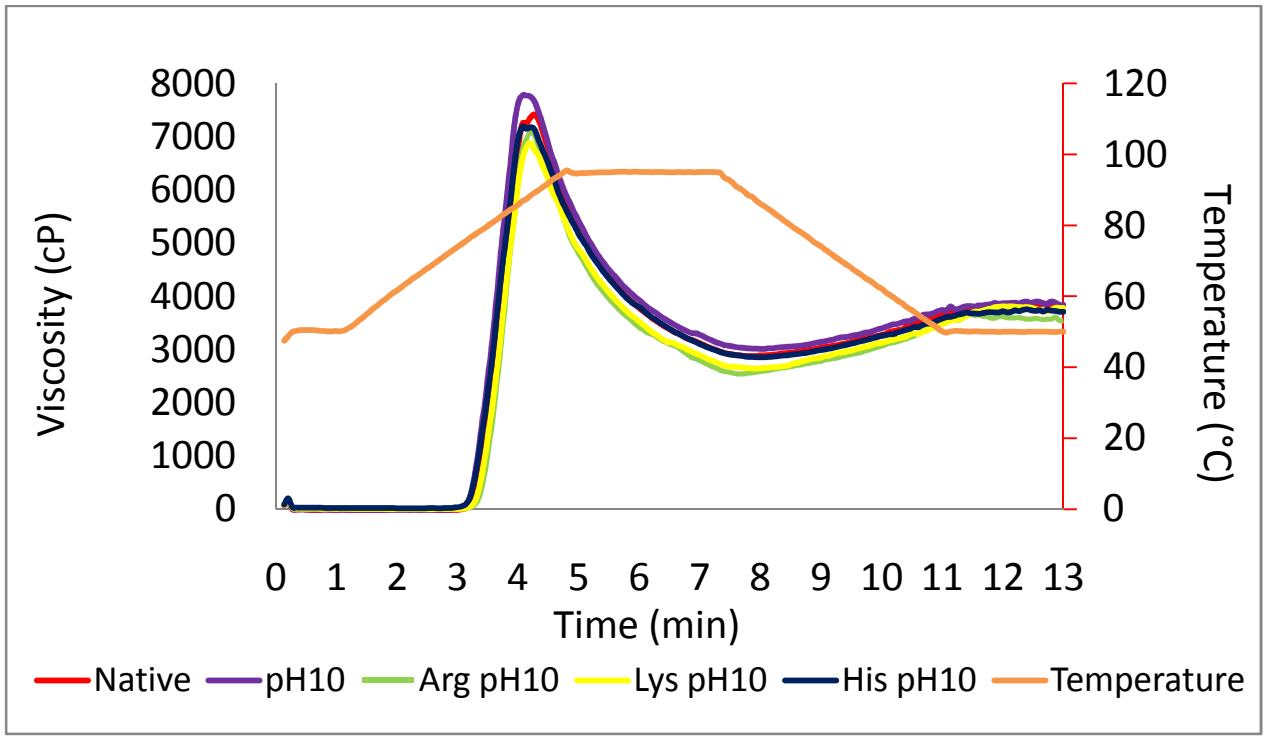

Figure 4.12. Pasting curve of Freeze-dried Evangeline Starch at pH 10 for 30 min

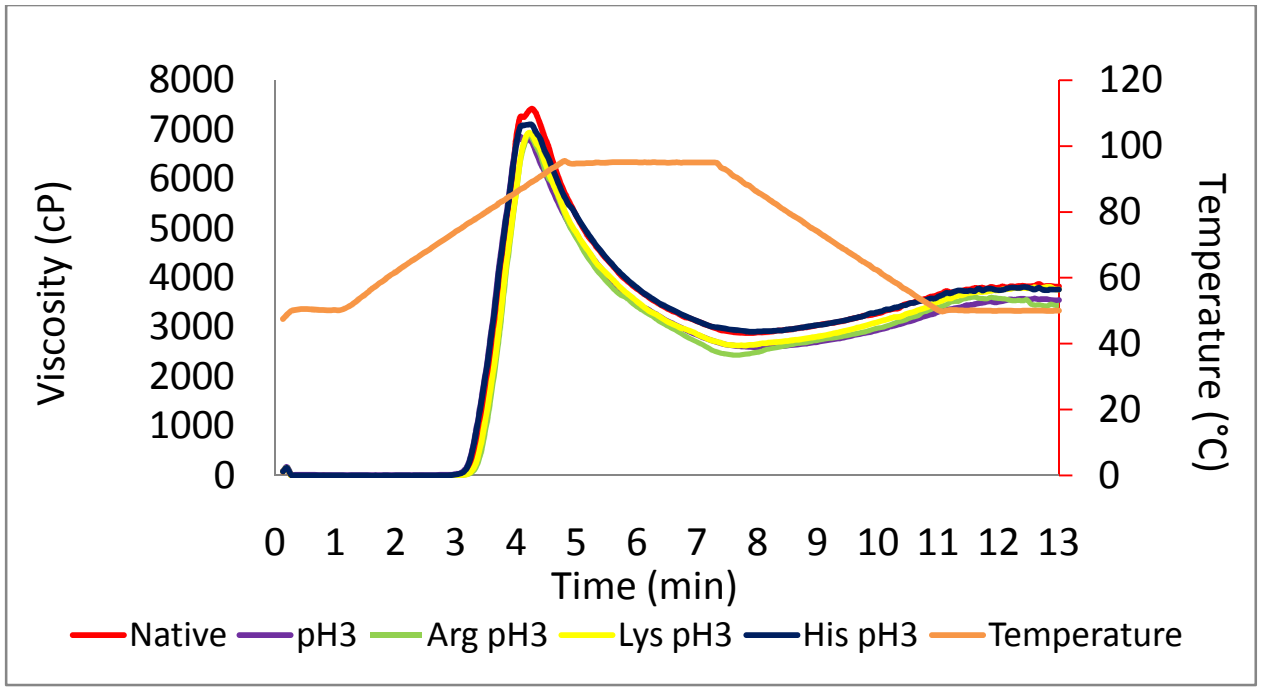

Figure 4.13. Pasting curve of Freeze-dried Evangeline Starch at pH 3 for 1 hour

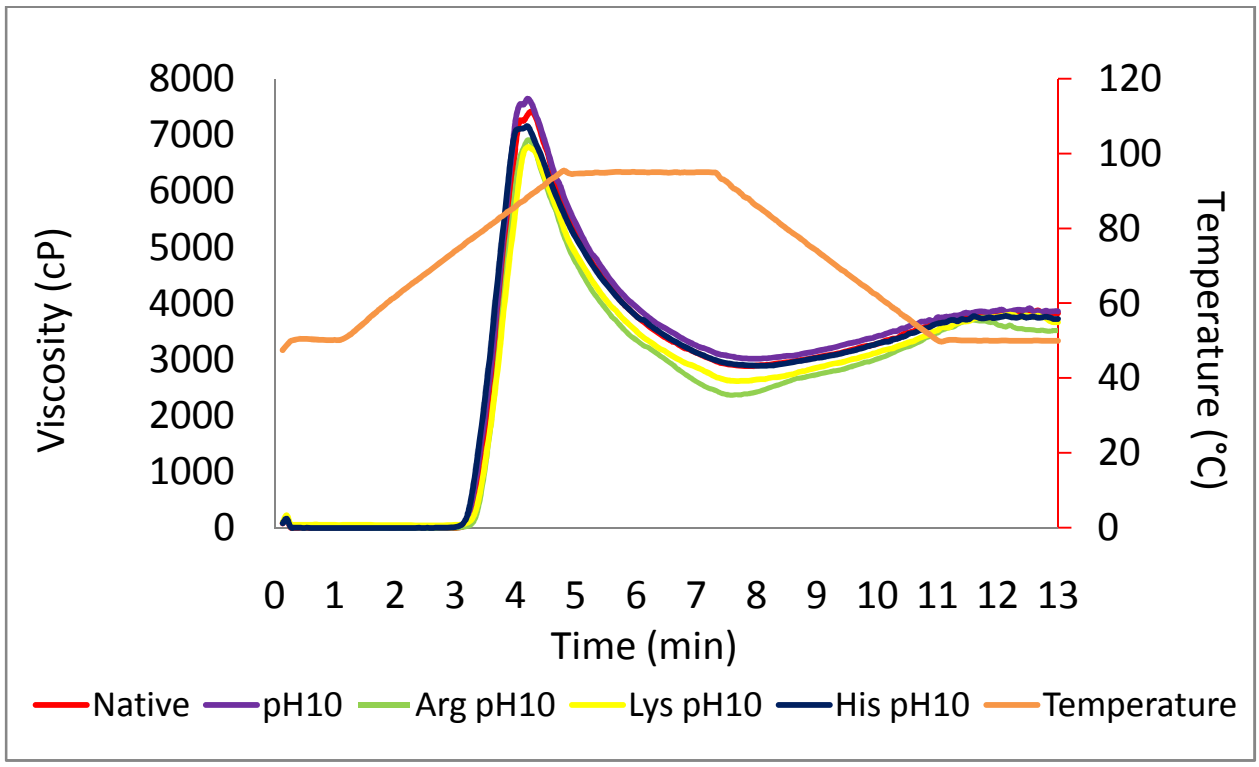

Figure 4.14. Pasting curve of Freeze-dried Evangeline Starch at pH 10 for 1 hour

For oven-dried Evangeline starch treated with amino acids, all amino acids decreased the PV, except for pH 10 alone (Table 4.5). The largest decrease in PV was found for lysine alone at 5036 cP, showing that the starch was modified into a thinner paste. Addition of arginine and lysine decreased MV in all treatments compared to control and native starches. This agrees with the results obtained by Liang and King (2003) on rice starch and Ito and others (2004) on potato starch who found that charged amino acids had a positive effect on pasting characteristics. The largest decrease in MV was found with arginine alone at 1526 cP, producing a starch that is easier to cook. Histidine had no effect in any treatments (Table 4.5). The largest decrease in BD was found with starch treated with histidine at 3122 cP, and lysine and histidine at pH 10 also decreased BD, this signifies that the paste will be more stable to shear during cooking compared to control and native starch. Anderson and others (2002) found that after a heat-moisture treatment breakdown decreased as heating times increased. The largest decrease in FV was found with lysine alone at 3060 cP. Histidine at any pH and pH treatment alone did not affect FV. TSB

was increased with the addition of arginine and lysine for all treatments compared to native and control starch. Histidine had no effect on TSB (Table 4.5). The largest increase in TSB was found with arginine alone 1637 cP, which indicated a higher possibility for retrogradation. Ishiguro and others (2000) studied the retrogradation of sweet potato starch and found that retrogradation of sweet potato starch was inhibited by extra-short chains of amylopectin (DP 10) and promoted by amylose and extremely long chains of amylopectin. Tp did not change. Pt increased with arginine and lysine at pH 3 and pH 10 compared to the native starch and control starches. Ito and others (2006) found that in potato starch the binding of the amino acids to the starch chain is what regulates the pasting behavior of the starch based upon the selected amino acid.

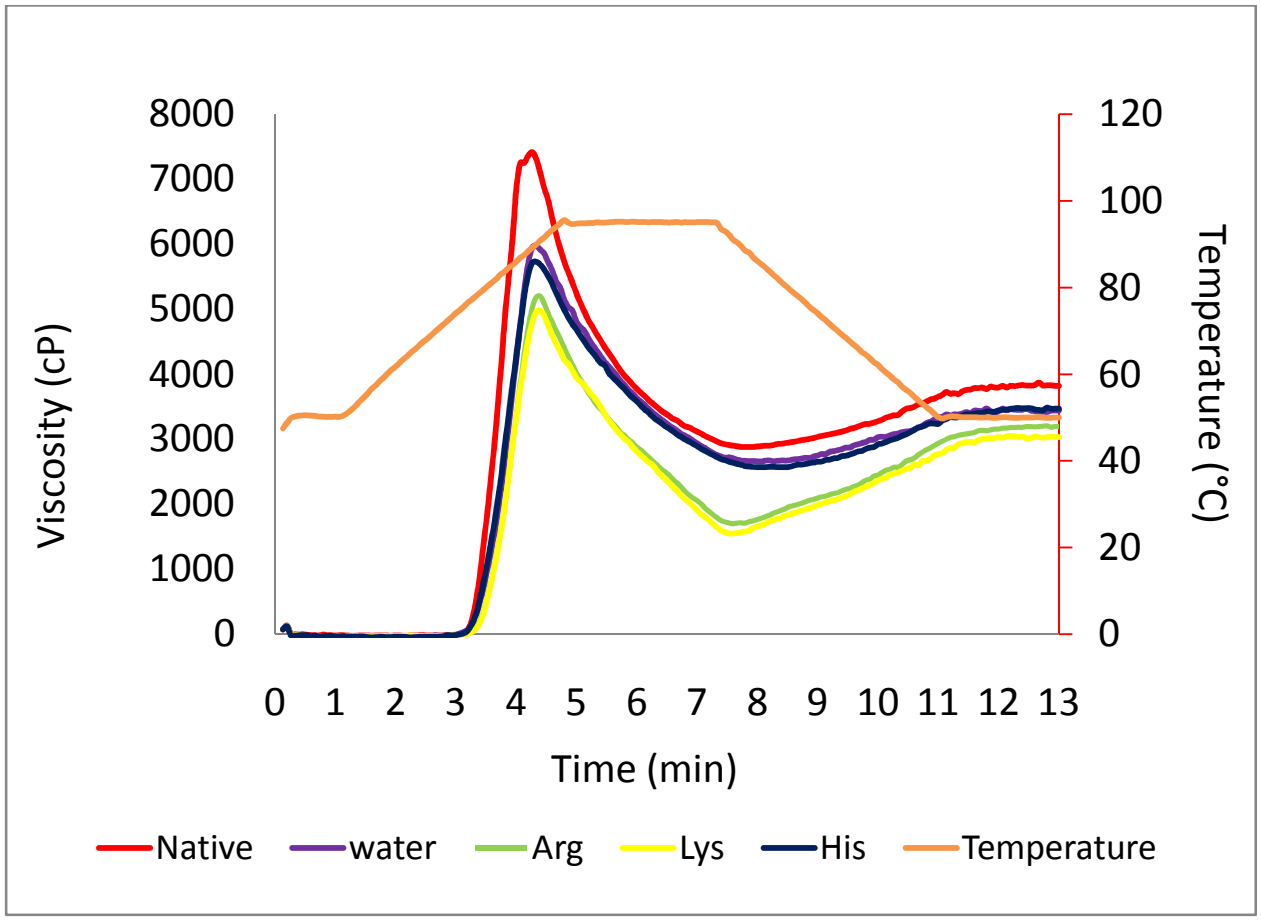

Figure 4.15. Pasting curve of Oven-dried Evangeline Starch with Amino Acids

Table 4.5. Pasting Properties Evangeline Oven-dried samples [1,2,3,4,5]

AA	pH	Time	PV	MV	BD	FV	TSB	Tp	Pt
Native			7458± 57.9a	2904± 33.9a	4554± 24.0a	3820± 0.71a	916± 34.7c	4.24± 0.05cd	74.03± 0.04bc
control	no	0min	6062± 128b	2661± 4.24ab	3401± 124bc	3475± 49.5b	814± 45.3c	4.3± 0.04abcd	73.63± 0.53c
Arg	no	0min	5140± 86.3fg	1526± 248e	3613± 161b	3163± 47.4fg	1637± 200a	4.37± 0.05ab	74.83± 0.04ab
Lys	no	0min	5036± 81.3g	1625± 105de	3411± 24.0bc	3060± 33.9g	1434± 71.4ab	4.4± 0.0a	74.78± 0.04ab
His	no	0min	5681± 67.9cd	2559± 9.9b	3122± 57.9de	3456± 15.6b	897± 5.7c	4.33± 0.0abc	73.98± 0.04bc
noaa	3	30min	5710± 7.07c	2501± 12.0b	3209± 5.66cde	3383± 9.2bc	882± 21.2c	4.20± 0.0d	74.48± 0.53bc
Arg	3	30min	5124± 60.8fg	1900± 46.7cd	3224± 14.1cde	3246± 60.1def	1346± 106.8b	4.27± 0.0bcd	75.55± 0.0a
Lys	3	30min	5274± 4.24efg	2036± 58.7c	3237± 62.9cde	3304± 29cd	1268± 29.7b	4.27± 0.0bcd	75.58± 0.04a
His	3	30min	5682± 6.4cd	2519± 6.36b	3163± 0.00cde	3453± 4.24b	933± 10.6c	4.20± 0.0d	74.40± 0.57bc
noaa	10	30min	5826± 0.7bc	2554± 13.4b	3272± 14.1cd	3476± 15.6b	921± 28.9c	4.20± 0.0d	74.88± 0.04ab
Arg	10	30min	5306± 1.4ef	1968± 26.2c	3337± 27.6cd	3300± 24.8cde	1332± 50.9b	4.27± 0.0bcd	75.58± 0.04a
Lys	10	30min	5071± 104fg	1943± 19.1c	3127± 85.6de	3183± 12.0ef	1240± 7.1b	4.30± 0.04abcd	75.60± 0.07a
His	10	30min	5452± 0.0de	2458± 0.00b	2994± 0.00e	3393± 0.00bc	935± 0.0c	4.20± 0.0d	74.05± 0.0bc

[1] Means in the same column with the same letter are not significantly different at $p \geq 0.05$

[2]Control is starch with water added prior specified drying method.

[3]Arg is arginine, Lys is lysine and His is histidine

[4]AA is amino acid, PV is peak viscosity, MV is minimum viscosity, BD is breakdown, FV is final viscosity, TSB is total setback, Tp is time to peak and PT is pasting temperature.

[5]Units = viscosity (cP); temperature (ºC); time (min.)

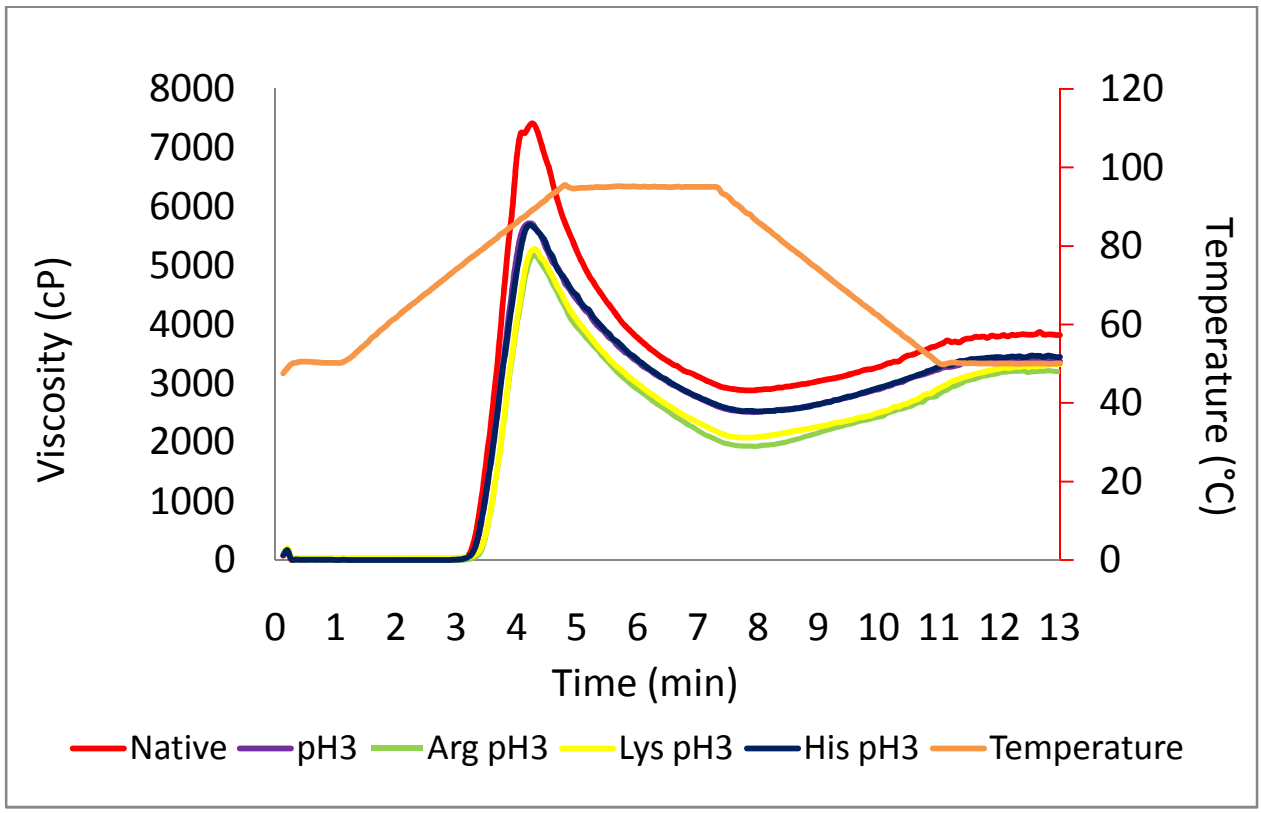

Figure 4.16. Pasting curve of Oven-dried Evangeline Starch at pH 3

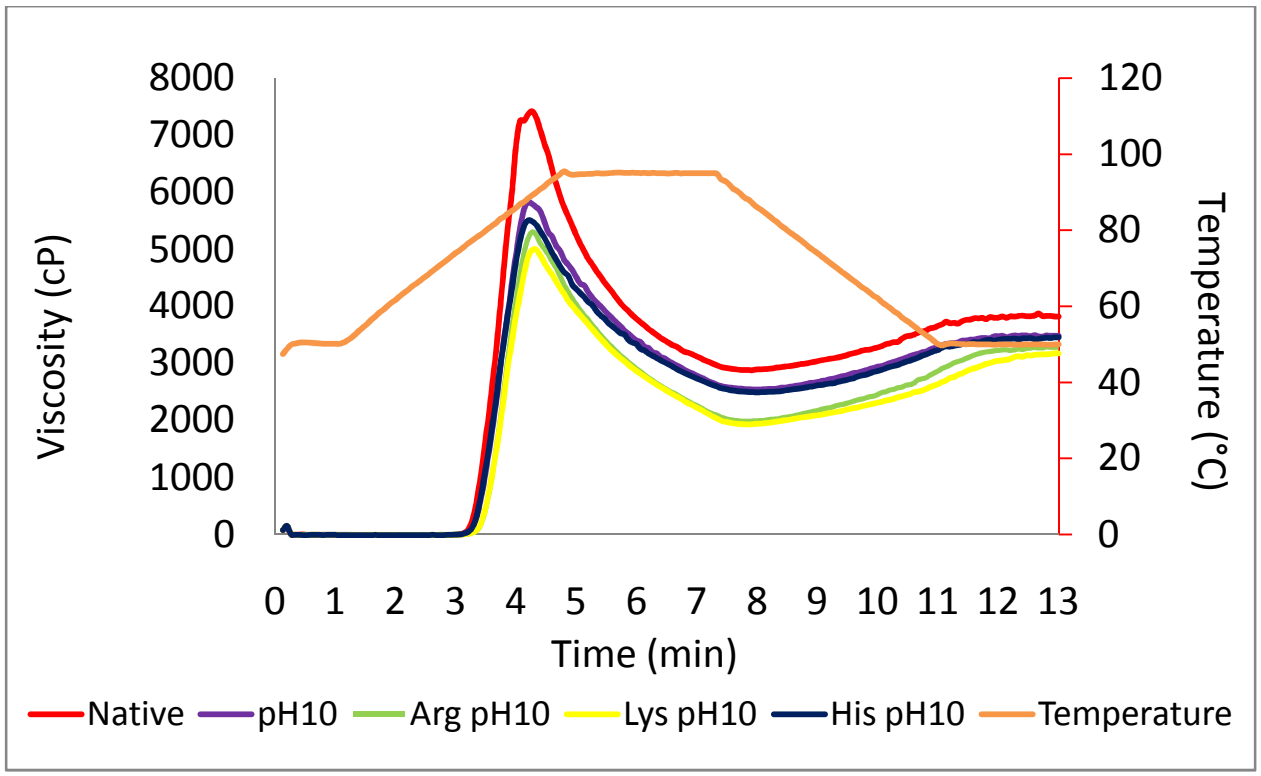

Figure 4.17. Pasting curve of Oven-dried Evangeline Starch at pH 10

4.3.4. Rheology Properties of Beauregard vs Evangeline Sweet Potato Starch

Measurements were performed to observe the changes in rheology of native Beauregard and Evangeline sweet potato starch. Figures 4.18 and Figure 4.19 show the changes in G' and G'' observed during heating the starch from 50 ºC to 95 ºC. Both moduli increased while temperature increased. The storage modulus G' was much greater than G" indicating that there were predominantly more elastic than viscous characteristic for both native Beauregard and Evangeline sweet potato starch. Singh and others (2008) studied the chain length of amylopectin between DP 6 and 30 for thermal and viscoelastic properties of potato starch. They found that potato starch having with a higher amount of short-chain amylopectin fractions (DP 6-12) showed more viscous characteristics than starches having a smaller amount of short-chain amylopectin fractions. Tan (δ) = G"/G' where, Tan (δ) quantifies the balance between energy loss and storage. G' is the energy recovered per cycle, the loss modulus G" is the energy dissipated per cycle and δ is mechanical material solid or liquid. With an increase in G' and a decrease in δ a more elastic material is displayed. (Tako and others 2009, Cho and others 2009) Moothy and others (2008) found that the rheological properties of tuber starches can be used to categorize them based upon their elasticity and viscosity.

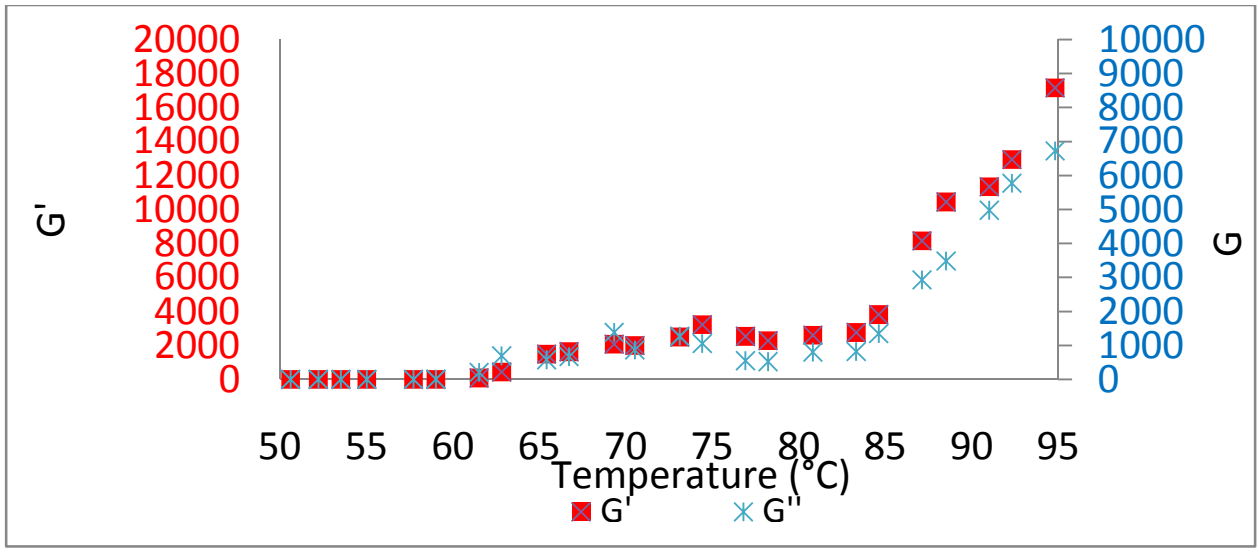

Figure 4.18. Rheology curve of Native Beauregard Sweet Potato Starch

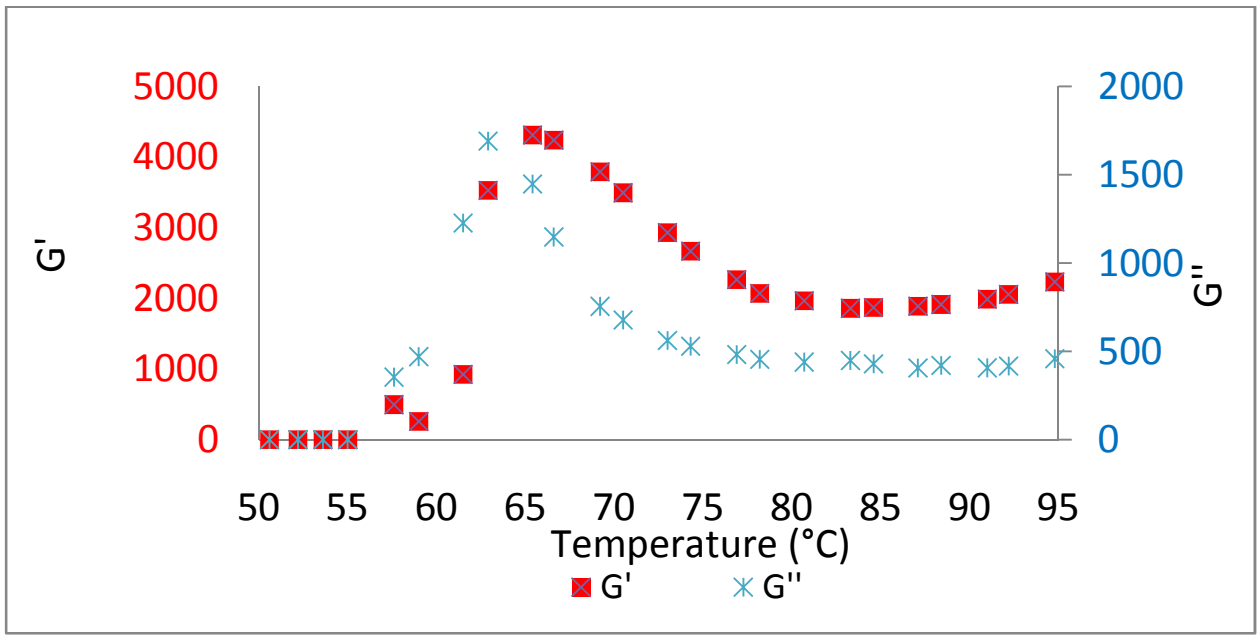

Figure 4.19. Rheology curve of Native Evangeline Sweet Potato Starch

4.4 Conclusion

This study showed that positively charge amino acids along with pH treatments are effective in altering the pasting properties of both Beauregard and Evangeline sweet potato starches. Oven-dried starch was more responsive to changes in pasting characteristics than freeze-dried sweet potato starch. Both arginine and lysine demonstrated a higher potential for retrogradation in Beauregard and Evangeline sweet potato starch. Breakdown could be maximally decreased by amino acids with pH treatment in Beauregard oven-dried starch making the starch more resistant to shear during cooking. In Evangeline oven-dried sweet potato starch, histidine lowered breakdown and with the addition of pH10 treatment breakdown decreased even further. The storage modulus G' was much greater than G" for both starches indicating that there were predominantly more elastic than viscous characteristic.

CHAPTER 5. EFFECT OF AMINO ACID AND pH TREATMENT ON FORMATION OF RESISTANT STARCH (RS) AND SLOWLY DIGESTABLE STARCH (SDS) ON BEAUREGARD AND EVANGELINE SWEET POTATO STARCH

5.1. Introduction

Resistant starch was first defined by Englyst in 1982 to describe a small fraction of starch that was resistant to hydrolysis by amylase and pullulanase treatments (in vitro). Resistant starch is the portion of starch that is not digested in the small intestine and passes into the colon where it can be fermented by natural micro-flora to short-chain fatty acids (Englyst and others 1982). There are three basic groups that starch can be classified into: rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) (Englyst and Cummings 1992). Resistant starch has physiological effects in the human body that are similar to that of dietary fiber, which have been shown to reduce the risks for some diseases, including colon cancer, coronary heart disease, and glycemia (Berry 1986). RS has a small particle size, white appearance, and bland flavor. It also has a low water-holding capacity.

Resistant starch is broken down into four different fractions: type I, type II, type III, type IV. Type I, is physically protected, found in partly milled grains and seeds. Type II is ungelatinized RS granules, found naturally in foods with type B crystallinity, slowly hydrolyzed by alpha-amyase. Type III, is formed by thermal disruption of the granular structure of the starch in water (gelatinization). Once the gel begins to cool re-crystallization of amylose and amylopectin takes place (also called retrogradation). Today most industrial production of resistant starch comes only from high amylose maize starches. Type IV, are chemically modified starches due to cross-linking with chemical reagents.

There are several different methods to measure resistant starch of an individual sample. The total dietary fiber method of the Association of Official Analytical Chemists AOAC method 991.43 and the AACC method 32-07 (AOAC 2000), are methods used to quantify for RDS, SDS and RS that can be determined using the Englyst method (Englyst and others 1992). The

Megazyme method (AOAC method 2002.02) focuses on enzymatic digestion at 37 °C and has been shown to be reproducible and repeatable.

Slowly Digestible Starch (SDS) defined by Engyst is the amount of starch that is likely to be completely digested in the small intestine between 20 min and 120 min (Englyst 1992). SDS can be used to physiologically benefit individuals with type 2 diabetes because it prevents hyperglycemia and hypoglycemia. It can also be used in food products that assist in weight loss and can be beneficial to athletes by providing a longer consistent source of systemic glucose (Wolf and other 1999).

Most research has centered on the production of Type III RS in a variety of different starches. Many methods that have been explored including but not limited to, partial acid hydrolysis, heat-moisture and temperature treatments, and enzymes (Shin and others 2004; Tan 2003).

Sweet potatoes are an excellent source of starch although starch modification has not been fully explored to test the potential of producing sweet potato resistant starch. The objectives of this study were 1) to compare RS properties of Beauregard and Evangeline sweet potato starches, 2) to examine whether there would be differences in the effects of positively charged amino acids on RS content of sweet potato starches, 3) to determine whether pH changes would alter RS content of positively charged amino acids and whether pH and amino acids can increase levels of RS in sweet potato starches.5.2. Materials and Methods

5.2.1. Materials

Starch was extracted from Beauregard and Evangeline sweet potatoes grown at the LSU AgCenter research station harvested in September of 2008. In this study positively charged amino acids were added with and without pH treatment at pH 3 and 10. The amino acids were purchased from Sigma Chemical Company (St. Louis, Missouri). Amino acids used include

positively charged arginine, lysine and histidine. These amino acids were chosen based upon past research (Liang 2001, An 2005 and Lockwood 2008).

5.2.2. Sweet Potato Starch Extraction

See Chapter 3 for details on starch extraction procedure

5.2.3. Starch Treatment

See Chapter 3 for details on starch treatment procedure

5.2.4. Resistant Starch Determination Procedure

Resistant starch content in each sample was determined by the Megazyme procedure (Megazyme International Ireland Limited, Bray, Ireland). The Megazyme method is an approved AOAC method (method 2002.02) and AACC method (method 32-40). The freeze-dried and oven-dried samples were ground using a mortar and pestle, and stored in airtight bags at room temperature. A 100 mg sample was weighed into a screw cap tube, and gently tapped to ensure that the entire sample fell to the bottom of the tube. Four milliliters of pancreatic alph-amylase (Pancreatin, 10 g, 3 Ceralpha Units/mg) (10 mg/mL) containing amyloglucosidase (AMG) (3 U/ mL) was then added. The tubes were tightly capped and mixed on a vortex mixer and attached horizontally in a shaking water bath. The tubes were incubated at 37 °C with continuous shaking for exactly 16 hours. Then the tubes were removed from the water bath and a paper towel was used to remove any excess water. The tube caps were removed and the contents treated with 4.0 mL of ethanol (99 %) then stirred on a vortex mixer. The tubes were centrifuged at 1500 x g (approximately 3000 rpm) for 10 min non-capped. All supernatants were decanted and the pellets were re-suspended in 2 mL of 50 % ethanol then stirred on a vortex mixer. A further 6 mL of 50% ethanol was added to the tubes then mixed on a vortex mixer and centrifuged again at 1500 x g for 10 min. Supernatants were decanted and then the suspension and centrifugation steps were repeated once more. The supernatants were carefully decanted and the tubes inverted on absorbent paper to drain excess liquid. A magnetic stirrer bar and 2 mL of 2M KOH were added

to each tube and the pellets were re-suspended by stirring for approximately 20 min in an ice/water bath over a magnetic stirrer. Eight mL of 1.2 M sodium acetate buffer (pH 3.8) were added to each tube with stirring on the magnetic stirrer. Immediately, 0.1 mL of AMG (300 U/ mL) was added and mixed well. The tubes were placed in a water bath at 50 °C. The tubes were incubated for 30 min with intermittent mixing on a vortex mixer. For samples containing <10 % resistant starch, the tubes were directly centrifuged at 1500 g for 10 min. For sample containing >10 % resistant starch, the contents of the tubes were transferred to a 100 mL volumetric flask with the use of a water wash bottle. The contents of the flask was adjusted to 100mL with distilled water and mixed well. An aliquot of this diluted sample was then centrifuged at 1500 g for 10 min. 0.1 mL aliquots of either the diluted or undiluted supernatants were transferred into glass test tubes, treated with 3.0 mL of Glucose Determination Reagent (GOPOD) and incubated at 50 °C for 20 min. A reagent blank was made by mixing 0.1mL of 0.1M sodium acetate buffer (pH 4.5) and 3.0 mL of GOPOD reagent. The absorbance of each solution was measured at 510 nm against the reagent blank. (AOAC method 2002.02)

The calculations for the percent of resistant starch were performed as follows:

Samples containing >10% resistant starch:

$= \Delta E*F*100/0.1*1/1000*100/W*162/180$

$= \Delta E*F/W*90$

Samples containing < 10 % resistant starch:

$= \Delta E*F*10.3/0.1*1/1000*100/W*162/180$

$= \Delta E*F/W*9.27$

Where:

ΔE = absorbance read against reagent blank

F = conversion form absorbance to micrograms (the absorbance obtained for 100 µg of glucose in the GOPOD reaction is determined and F = 100 µg of glucose divided by the GOPOD absorbance for this 100 µg of glucose)

100/0.1 = volume correction (0.1 mL taken from 100 mL)

1/1000 = conversion for micrograms to milligrams

W = dry weight of sample analyzed

100/W = factor to present RS as a percentage of sample weight

162/180 = factor to convert from free glucose, as determined, to anhydro-glucose as occurs in starch

10.3/0.1 = volume correction (0.1 mL taken from 10.3 mL) for samples containing 0-10% RS where the incubation solution is not diluted and the final volume is about 10.3mL

5.2.5. Slowly Digestible Starch Determination Procedure

A sample of 0.8 g was weighed into a 50 ml polypropylene tube to the nearest 0.1 mg. Fifty mg of guar gum and 5 glass balls were added, followed by 20 ml 0.1M acetate buffer and mixed. The samples, standards and blank were equilibrated in a 37 °C water-bath. One tube at a time was removed and immediately 5 ml Enzyme Solution 1 (diluted AMG 140 U/mL, pancreatin 8x and invertase 30,000 U/g) was added. Tubes were capped and immersed horizontally in a 37 °C shaking water-bath. After 20 min, 0.5 mL of sample was removed and placed into a labeled (G_{20}) tube containing 20ml 66 % ethanol and mixed well. Immediately the sample tubes were replaced in a 37°C shaking water-bath. After a further 100 min (total now of 120 min), a second 0.5 ml of sample was removed and placed into a labeled (G_{120}) tube. The G_{20} and G_{120} portions were centrifuged for 1-2 min to obtain a clear supernatant. The glucose in these portions was measured, using the following values: V_t =25 plus 1ml per gram wet weight of sample used, C=20 and D=1.

$$\% \text{ glucose} = [A_t * V_t * C * D / A_s * W_t] * 100 \qquad \% \text{ glucose} = [A_t * 25 * 20 * 1 / A_s * W_t] * 100$$

Where: A_t –is absorbance of test solution, V_t –is total volume of test solution, C – is concentration (in mg glucose/ml) of standard, D – is a dilution factor, A_s – is absorbance of standard, W_t –is weight (in mg) of sample taken for analysis.

V_t –is total volume of the hydrolysate from which the subsample taken for glucose determination originates. C is the concentration of glucose in the standard solution treated identically to the hydrolysate from which the subsample is taken, unless the samples and standards are diluted differently, in which case a dilution factor (D) is introduced. The calculation of these constants takes account of the subsamples taken during the procedure.

$$SDS = (G_{120} - G_{20}) * 0.9$$

5.2.6. Statistical Analysis

SAS (Statistical Analysis System) software (version 9.1) was used to analyze the RS and SDS data. Standard deviation, ANOVA (Analysis of Variance), and Tukey's Studentized Range (HSD) were used to examine the effects of the amino acid additives on the formation of RS and SDS of the sweet potato starches, on a $p \leq 0.05$ level.

5.3. Results and Discussion

5.3.1. The Effect of Amino Acids, pH and Time on Beauregard Sweet Potato Starch RS Levels

The native Beauregard sweet potato starch had 10.6 % RS (Table 5.1). The freeze-dried Beauregard sweet potato starch samples were not significantly altered by pH treatments. Lysine was the only treatment that significantly increased the resistant starch content compared to the native starch, but was not different from control. An (2005) found that lysine alone significantly affected resistant starch percentage in rice starch.

The native Beauregard sweet potato starch was effectively altered by oven-drying starch samples after combined pH and amino acid treatments (Table 5.2). Arginine increased the RS content of native Beauregard starch from 10.6 % to 12.11 %. The addition of arginine at pH10

increased RS content significantly to 17.66 % compared to native and control starch. Lysine increased the RS content to 12.07 % compared to native starch. With lysine at pH3, RS increased to15.85 % and at pH 10 lysine increased RS to 16.66 %. Histidine increased the RS content at pH3 to 16.24 % and at pH 10 to 16.1 %. Beauregard sweet potato starch was altered with a change in pH alone, as pH 3 and pH 10 significantly increased the RS content to 13.95 % and 15.92 %, respectively, compared to native and control starch. Edmonton and Saskatoon (1998) found that acid treatments increased RS3 content of annealed gel. Shin and others (2004) found that partial acid-hydrolysis with heating-cooling cycles followed by a heat-moisture treatment increased RS content significantly.

5.3.2. The Effect of Amino Acids, pH and Time on Evangeline Sweet Potato Starch RS Levels

The native Evangeline sweet potato starch had a resistant starch value of 12.22 % (Table 5.1). Most of the amino acid additives and pH treatments were not effective in altering the resistant starch content of freeze-dried Evangeline sweet potato starch samples. Arginine and lysine alone did significantly increase resistant starch content compared with the native and control to 14.12 % and 13.73 %, respectively.

The native Evangeline sweet potato starch, was effectively altered by oven-drying starch samples after the addition of amino acids and pH treatments (Table 5.2). Arginine and histidine increased RS content to 15.48 % and 14.81 %, respectively, while histidine at pH10 increased RS content to 18.58 %. Lysine by itself did not significantly increase RS content but lysine at pH 3 and pH 10 increased the RS content to 15.43 % and 18.92 %, respectively. The Evangeline sweet potato starch treated at pH 3 and pH 10 alone significantly increased the RS content to 15.97 % and 17.98 %, respectively. There was no significant increase in RS between samples treated with pH alone and samples treated with amino acids at pH 3 or pH 10. Escarpa and others (1996) found higher RS yields with high-pressure heat treatment of potato starch.

The amylose content of Evangeline sweet potato starch (Chapter 3, Table 3.1) was higher than the Beauregard sweet potato starch. The amylose contents were 27.1 % for Evangeline starch, while only 23.6 % for Beauregard starch. Escarpa and others (1996) found higher resistant starch yields from starch containing high amylose contents. Oven-dried sample had a noticeable higher RS content than freeze-dried samples (Table 5.1 and Table 5.2). Sajilata (2006) found that amylose content is the main component for retrograded starch which is found after heating and cooling cycles.

Table 5.1. Resistant Starch Values for Freeze-dried Beauregard and Evangeline Sweet Potato Starch[1,2,3,4]

Freeze-dried			Beauregard	Evangeline
AA	**pH**	**Time**	**RS%**	**RS%**
Native	no	0min	10.55±0.28 bcde	12.22±0.10cde
Control	no	0min	11.65±1.56 abcd	13.24±0.5abcde
Arg	no	0min	13.17±1.48 ab	14.12±1.88ab
Lys	no	0min	13.61±1.34 a	13.73±0.31ab
His	no	0min	12.09±0.69 abcd	12.91±0.36abcde
noaa	3	30min	9.45±0.22 de	11.5±0.91e
Arg	3	30min	10.97±0.25 abcde	12.97±0.53abcde
Lys	3	30min	11.73±0.31 abcd	12.17±0.9cde
His	3	30min	10.69±0.32bcde	12.59±0.8abcde
noaa	10	30min	9.65±1.55 cde	14.15±0.69a
Arg	10	30min	11.3±0.31 abcde	12.63±0.44abcde
Lys	10	30min	10.86±0.81 bcde	11.94±0.32de
His	10	30min	10.34±0.599 cde	12.53±0.28abcde
noaa	3	1hour	9.77±0.58 cde	12.54±0.67abcde
Arg	3	1hour	11.4±0.78 abcde	14.06±0.46abc
Lys	3	1hour	12.3±1.44 abc	12.23±0.32bcde
His	3	1hour	11.65±1.14 abcd	13.74±1.2abcd
noaa	10	1hour	8.89±0.47 e	13.22±0.65abcde
Arg	10	1hour	11.69±1.53 abcd	13.57±0.51abcd
Lys	10	1hour	11.57±1.45 abcd	13.64±0.67abcd
His	10	1hour	11.81±1.29 abcd	13.45±0.25abcd

[1]* Means in the same column with the same letter are not significantly different at $p \geq 0.05$

[2]*Control is starch with water added prior specified drying method.

[3]*Arg is arginine, Lys is lysine and His is histidine

[4]*AA is amino acid, RS% is the value of resistant starch.

Table 5.2. Resistant Starch Values for Oven-dried Beauregard and Evangeline Sweet Potato Starch [1,2,3,4]

Oven-dried		Beauregard	Evangeline
AA	**pH**	**RS%**	**RS%**
Native	no	10.6±0.34 d	12.67±0.36 e
Control	no	10.89± 0.38 cd	13.32±0.39 e
Arg	no	12.11±0.59 c	15.48±0.29 b
Lys	no	12.07±0.39 c	13.56±0.73 cde
His	no	11.36±0.66 cd	14.81±0.52 bc
noaa	3	13.95±0.53 b	15.97±0.21 b
Arg	3	11.86±0.78 c	15.45±0.83 b
Lys	3	15.85±0.83 ab	15.43±1.02 bc
His	3	16.24±0.77 ab	16.28±0.84 bc
noaa	10	15.92±0.58 ab	17.98±0.58 a
Arg	10	17.66±0.28 a	12.2±0.56 e
Lys	10	16.66±0.83 a	18.92±0.84 a
His	10	16.1±0.47ab	18.58±0.38 a

[1]* Means in the same column with the same letter are not significantly different at p≥0.05

[2]*Control is starch with water added prior specified drying method.

[3]*Arg is arginine, Lys is lysine and His is histidine

[4]*AA is amino acid, RS% is the value of resistant starch.

5.3.3. The Effect of Amino Acids, pH and Time on Beauregard Sweet Potato Starch SDS Levels

The native Beauregard sweet potato starch had an SDS value of 5.72 % (Table 5.3). All treatments for freeze-dried Beauregard starches, with the exception of lysine at pH10 for 30 min, altered the SDS levels. Arginine alone increased SDS content to 13.63 %, arginine at pH 3 for 30 min decreased SDS to 11.44 % and at 1 hour decreased SDS to 2.14 %. Arginine at pH 10 decreased SDS for 30 min and 1 hour to 4.26 % and 1.32 %, respectively. Lysine alone decreased SDS content to 3.84 %, while lysine at pH 3 for 30 min and 1 hour increased SDS to 11.99 % and 13.77 %, respectively. Lysine at pH10 decreased the SDS value of Beauregard sweet potato starch. Histidine alone decreased SDS value compared to the native and control Beauregard starch, however histdine at pH 10 for 30 min and 1 hour the SDS content increased

to 15.27 % and 7.52 %, respectively. Histidine at pH 3 after 1hour did increase the SDS content compared to the native and control Beauregard starch to 14.68 %.

The oven-dried Beauregard sweet potato starch SDS content increased with the addition of water (control) from native 5.72 % to control 8.17 % (Table 5.4). The addition of arginine alone significantly increased the SDS value to 11.74 % and arginine at pH 3 to 15.43 %. Lysine alone decreased the SDS level but when combined with pH 3 SDS increased to 9.65 %. Han and BeMiller (2007) found that acetylation (adjusting pH to 8.0-8.4 and adding acetic anhydride for 10 min before neutralizing the starch) increased SDS production. Histidine had little effect on SDS content except having a significant decrease with pH 10 to 0.96 %. Both pH 3 and pH 10 alone increased the SDS content compared with native and control Beauregard sweet potato starch to 10.10 % and 10.86 % respectively.

5.3.4. The Effect of Amino Acids, pH and Time on Evangeline Sweet Potato Starch SDS Levels

The native Evangeline sweet potato starch had an SDS value of 2.45 % (Table 5.3). Zhang and others (2006) found that potato starch had lower SDS content compared with cereal starches because of their A-type crystalline structure and higher amount of short A chains with a degree of polymerization (DP) of 5 to 10. When treated at pH 10 with arginine for 30 min and 1 hour SDS content significantly increased compared to native and control starch to 6.81 % and 4.49 %, respectively. Lysine decreased SDS content compared to the native starch to 1.33 %. When starch was treated with lysine at pH3 for 30 min and 1 hour SDS increased to 8.15% and 5.64%, respectively. With lysine added at pH10 for 1 hour SDS decreased to 3.91%. Freeze-dried Evangeline starch treated with histidine increased SDS value compared to the native starch. Histidine alone increased SDS to 6.23 %, and when treated with histidine at pH 3 for 30 min to 11.78 %. Histidine at pH10 for 30 min, pH 3 for 1hour and pH 10 for 1 hour increased SDS

content to 5.63 %, 6.26 % and 6.43 %, respectively. There were significant differences between pH treated samples and combined pH and amino acid treated samples.

Table 5.3. SDS Values Freeze-dried Beauregard and Evangeline Sweet Potato Starch. [1,2,3,4]

AA	pH	Time	Beauregard (% SDS)	Evangeline (% SDS)
Native	no	no	5.72±1.0g	2.45±0.14gi
Control	no	no	9.37±0.12e	0.39±0.12l
Arg	no	no	13.63±0.48 c	1.16±0.06kl
Lys	no	no	3.84±0.41h	1.33±0.34k
His	no	no	4.30±1.14h	6.23±0.62de
no	pH3	30min	7.58±0.21f	1.56±0.08jk
Arg	pH3	30min	11.44±0.28d	0.76±0.05kl
Lys	pH3	30min	11.99±0.16d	8.15±0.14c
His	pH3	30min	4.17±0.36h	11.78±0.47a
no	pH10	30min	11.62±0.42d	5.33±0.29fg
Arg	pH10	30min	4.26±0.33h	6.81±0.58d
Lys	pH10	30min	6.6±0.17fg	2.31±0.15ij
His	pH10	30min	15.27±0.22a	5.63±0.23ef
no	pH3	1 hour	14.86±0.54ab	2.69±0.12i
Arg	pH3	1 hour	2.14±0.09ij	0.32±0.24l
Lys	pH3	1 hour	13.77±0.4bc	5.64±0.25ef
His	pH3	1 hour	14.68±0.45abc	6.26±0.34de
no	pH10	1 hour	1.77±0.34ij	10.46±0.26b
Arg	pH10	1 hour	1.32±0.37j	4.49±0.35h
Lys	pH10	1 hour	2.54±0.26i	3.91±0.39gh
His	pH10	1 hour	7.52±0.32f	6.43±0.55de

[1] Means in the same column with the same letter are not significantly different at $p \geq 0.05$

[2]Control is starch with water added prior specified drying method.

[3]Arg is arginine, Lys is lysine and His is histidine

The oven-dried Evangeline sweet potato starch SDS content increased with the addition of water (control) from native 2.45 % to control 10.18 % (Table 5.4). Arginine alone did not

have a significant effect on SDS content. Arginine at pH 3 treatment significantly decreased the SDS value to 1.62%, however at pH10 SDS significant increased to 5.55% compared to native starch. Starch treated with lysine did not significantly alter the SDS value compared with the native Evangeline starch. Lysine at pH 3 increased SDS to 10.35% and at pH10 increased SDS to 3.96%, compared to native starch. Histidine alone cause a slight increase to 3.52% but, increased SDS more at pH 3 and pH 10 to 6.17% and 5.86%, respectively. Evangeline starch treated at pH 3 had decreased SDS content to 1.49% and pH 10 increased the SDS content to 9.16% compared with the native Evangeline sweet potato starch.

Table 5.4. SDS Values Oven-dried Beauregard and Evangeline Sweet Potato Starch.[1,2,3,4]

AA	pH	Beauregard (% SDS)	Evangeline (% SDS)
Native	no	5.72±1.00g	2.45±0.14e
Control	no	8.17±0.32e	10.18±0.33a
Arg	no	11.74±0.15b	2.74±0.22e
Lys	no	1.90±0.45h	2.35±0.3ef
His	no	5.39±0.17g	3.52±0.16d
no	pH3	10.10±0.3cd	1.49±0.17g
Arg	pH3	15.43±0.51a	1.62±0.3fg
Lys	pH3	9.65±0.32d	10.35±0.12a
His	pH3	5.41±0.39g	6.17±0.37c
no	pH10	10.86±0.1bc	9.16±0.16b
Arg	pH10	7.06±0.66ef	5.55±0.57c
Lys	pH10	6.14±0.5fg	3.96±0.42d
His	pH10	0.96±0.26h	5.86±0.24c

[1] Means in the same column with the same letter are not significantly different at $p \geq 0.05$

[2]Control is starch with water added prior specified drying method.

[3]Arg is arginine, Lys is lysine and His is histidine

The SDS levels between Beauregard and Evangeline sweet potato starch were noticeably different. Native Beauregard starch had a higher SDS content of 5.72 % compared to native

Evangeline starch having an SDS content of 2.45 %. Both native starches reacted differently when treated with water (used as a control) and the drying method was a significant factor for altering SDS values. Wolf and others (1999) found that chemically modified starches had small SDS values, but the modifications caused an increase in RS levels. The amylose content of Evangeline sweet potato starch was 27.1 % (Chapter 3, Table 3.1) which is higher than the Beauregard sweet potato starch of 23.6 %. Zhang and others (2006) found that SDS content is contributed by both amylopectin and amylase that are packed tight and are therefore less susceptible to enzymatic digestion.

5.4. Conclusion

This study showed that oven-dried Beauregard and Evangeline sweet potatoes starch were more responsive to pH changes for increasing resistant starch content than the freeze-dried samples. When both oven-dried Beauregard and Evangeline starch were treated at pH3 and pH10 an increase in RS formation was found. There was a trend towards increased RS with amino acids added at pH3 or pH10 versus pH3 or pH10 alone, especially for lysine and histidine.

SDS content increased for freeze-dried Beauregard starch with arginine alone, arginine at pH3 for 30 min, pH10 alone for 30 min and pH 3 alone for 1 hour. Histidine at pH10 for 30 min caused the highest increased SDS content for freeze-dried Beauregard sweet potato starch to 15.27%. pH3 for 1 hour and lysine and histidine at pH3 for 1 hour caused a large increase in SDS content. Oven-dried Beauregard starch had increased SDS content with arginine alone and in all pH amino acid combined treatments, with the exception of histidine. Freeze-dried Evangeline starch SDS content increased with histidine alone and all pH amino acids combined treatments, with the exception of pH3 and arginine at pH3 for 30 min and 1 hour and lysine at pH10 for 30 min. Oven-dried Evangeline starch SDS content increased by histidine alone, lysine and histidine at pH3 and all pH10 treatments.

Increasing RS and SDS levels in starch would improve the quality of starch by decreasing digestibility. With higher levels of RS and SDS the majority of the starch would pass all the way through the small intestine without being digested at all or be delayed so that most of the starch is not converted to glucose. Oven-drying was the more effective method for increasing RS levels of the starch. For the Evangeline starch lysine and histidine at pH10 increased RS levels the most and for Beauregard arginine at pH10. SDS levels seemed to increase more with oven-drying by observing the increase in control. Beauregard starch had the largest increased SDS level with arginine at pH3. Freeze-dried Evangeline starch with histidine at pH3 for 30 min increased SDS levels the most.

CHAPTER 6. GENERAL CONCLUSIONS AND RECOMMENDATIONS

There were several differences found after comparing Beauregard and Evangeline sweet potato starch by a t-test. Beauregard sweet potato starch had a lower onset temperature than Evangeline sweet potato starch. Both starches showed similar gelatinization temperatures. Beauregard starch required more energy than Evangeline to complete gelatinization. Beauregard had a lower amylose content than Evangeline sweet potato starch, however Beauregard had a higher tendency for retrogradation found using RVA. Beauregard sweet potato starch was found to be easier to cook, had a shorter cooking time and more swelling at higher temperatures. The native Evangeline sweet potato starch had a thinner paste and was more stable to shear during cooking. The Evangeline starch had a higher resistant starch, while Beauregard starch had a higher SDS content.

Gelatinization temperatures were decreased for Beauregard freeze-dried starch with pH treatments. pH10 and histidine at pH10 cause the largest decrease gelatinization temperature. Gelatinization temperatures for Evangeline freeze-dried starch were decreased by pH3 alone and histidine treatments. Beauregard oven-dried starch was more responsive to pH and amino acids treatments than Evangeline starch for decreasing gelatinization temperature.

Positively charge amino acids along with pH treatments caused significant alterations in pasting properties of both Beauregard and Evangeline sweet potato starches. The oven-dried starch was more responsive to changes in pasting characteristics than freeze-dried sweet potato starch. Both arginine and lysine demonstrated a higher potential for retrogradation in Beauregard and Evangeline sweet potato starch. Amino acids with pH treatment decreased BD in Beauregard oven-dried starch making the starch more resistant to shear during cooking. In Evangeline oven-dried sweet potato starch, histidine alone lowered breakdown and with histidine at pH10 treatment breakdown decreased even further, increasing its stability to shear during cooking.

Oven-dried Beauregard and Evangeline starch treated at pH3 and pH10 increased RS. There was a trend towards increased RS with amino acids added at pH3 or pH10 versus pH3 or pH10 alone, especially for lysine and histidine.

There are trends in the relationship between an increase in the DCS gelatinization temperature and an increase in RVA Tp however; there were no significant increases in the DSC or RVA samples that would show a positive correlation between the two. There was a trend in TSB and formation of RS found in treatments. As RS increased, TSB increased for freeze-dried Evangeline and oven-dried Beauregard starch for arginine and lysine treatments. Oven-dried Evangeline starch increased in RS and TSB with arginine, arginine and lysine at pH3 and lysine at pH10.

SDS content increased for freeze-dried Beauregard starch with arginine alone and at pH3. Histidine at pH10 for 30 min caused the highest increased SDS content for freeze-dried Beauregard sweet potato starch. SDS showed large increases with pH3 for 1 hour and lysine and histidine at pH3 for 1 hour. SDS content increased in oven-dried Beauregard starch the most with arginine at pH3. Freeze-dried Evangeline starch SDS content increased the greatest with histidine at pH3. Freeze-dried Evangeline starch SDS content also increased with histidine alone and all pH amino acids combined treatments, with the exception of pH3 and arginine at pH3 for 30 min and 1 hour and lysine at pH10 for 30 min. SDS content increased the highest with lysine at pH3 for oven-dried Evangeline starch. The increases in SDS levels of starch provide a healthier starch option which may be suitable for those fighting diabetes, cardiovascular disease and obesity.

Overall, the addition of positively charged amino acids caused changes in both Beauregard and Evangeline sweet potato starches. The amino acids alterations could be further charged with the addition of pH treatments. The method of drying the starch was significant especially for increasing resistant starch content. More research should be done to determine how

or where amino acids attach to the starch. Also, other drying methods could be done to examine if starch properties are affected.

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APPENDIX 1: DSC RAW DATA AND SAS CODE

Freeze-dried Beauregard DCS data

AA	pH	Time	Rep	Onset	Peak	Conclusion	Enthalpy
Native B	no	0min	1	57.73	73.96	83.97	9.233
Native B	no	0min	2	56.53	74.3	84.26	8.635
control	no	0min	1	45.93	73.56	84.99	14.18
control	no	0min	2	47.74	73.34	85.04	14.67
Arg	no	0min	1	60	75.59	86.39	7.189
Arg	no	0min	2	53.18	74.68	87.17	12.24
Lys	no	0min	1	50.87	75.51	83.97	12.85
Lys	no	0min	2	49.9	74.82	84.67	13.88
His	no	0min	1	52.73	73.54	82.25	9.42
His	no	0min	2	58.11	73.8	80.24	5.039
noaa	3	30min	1	61.15	70.96	84.03	8.065
noaa	3	30min	2	50.07	70.29	84.06	14.93
Arg	3	30min	1	58.39	72.38	88.06	11.43
Arg	3	30min	2	52.57	71.99	84.87	14.52
Lys	3	30min	1	61.37	72.45	85.45	10.41
Lys	3	30min	2	58.22	73.51	92.13	14.8
His	3	30min	1	60.33	70.51	79.71	9.721
His	3	30min	2	56.33	70.44	83.26	12.5
noaa	10	30min	1	63.76	71.6	83.66	5.979
noaa	10	30min	2	60.29	71.26	82.41	9.705
Arg	10	30min	1	62.56	72.52	83.96	9.679
Arg	10	30min	2	64.28	72.52	83.29	8.159
Lys	10	30min	1	59.57	72.17	84.41	11.98
Lys	10	30min	2	61.59	72.08	81.51	9.641
His	10	30min	1	60.4	70.59	81.05	9.492
His	10	30min	2	60.98	71.05	83.88	8.595
noaa	3	1hour	1	56.67	70.51	84.12	13.55
noaa	3	1hour	2	63.83	70.82	81.42	6.902
Arg	3	1hour	1	63.61	72.04	80.9	8.31
Arg	3	1hour	2	57.13	72.07	86.56	13.11
Lys	3	1hour	1	65.4	73.09	83.29	6.884
Lys	3	1hour	2	62.34	71.52	80.68	8.626
His	3	1hour	1	58.74	70.51	81.47	11.11
His	3	1hour	2	60.53	69.85	81.02	8.801
noaa	10	1hour	1	57.58	68.87	79.95	11.36
noaa	10	1hour	2	55.52	68.58	80.93	12.59
Arg	10	1hour	1	62.32	71.78	81.47	8.838
Arg	10	1hour	2	63.9	71.02	81.95	8.852
Lys	10	1hour	1	59.01	71.84	83.26	11.77
Lys	10	1hour	2	58.39	70.45	82.9	13.18
His	10	1hour	1	59.5	70.24	80.22	10.63
His	10	1hour	2	57.86	69.12	82.23	10.79

Freeze-dried Evangeline DSC data

AA	pH	Time	Rep	Onset	Peak	End	Enthalpy
Native E	no	0	1	65.31	75.8	86.39	5.436
Native E	no	0	2	68.23	75.12	83.34	3.104
Control	no	0	1	64.29	73.93	82.18	5.242
Control	no	0	2	64.65	74.68	81.64	4.219
Arg	no	0	1	69.57	75.89	82.77	2.686
Arg	no	0	2	69.2	75.76	82.02	2.978
Lys	no	0	1	69.65	76.48	83.14	2.874
Lys	no	0	2	68.39	76.73	83.33	3.233
His	no	0	1	65.59	75.16	83.24	3.73
His	no	0	2	67.26	74.97	82.77	3.012
no	3	30	1	61.15	70.96	84.03	8.065
no	3	30	2	63.85	73.67	82.74	8.433
Arg	3	30	1	59.99	74.92	85.04	11.59
Arg	3	30	2	61.76	74.69	85.04	11.13
Lys	3	30	1	55.61	75.22	88.27	14.23
Lys	3	30	2	63.61	75.12	83.44	9.742
His	3	30	1	55.43	73.46	85.67	14.58
His	3	30	2	55.97	73.18	83.61	12.79
no	10	30	1	57.4	73.65	82.99	12.35
no	10	30	2	63.01	73.36	82.39	9.088
Arg	10	30	1	50.67	74.14	86	15.33
Arg	10	30	2	63.73	74.32	83.95	9.345
Lys	10	30	1	65.92	74.93	83.14	7.943
Lys	10	30	2	65.77	74.94	86.49	9.034
His	10	30	1	58.08	73.34	83.58	12.3
His	10	30	2	64.21	73.42	84.3	8.185
no	3	60	1	65.62	74.6	82.57	7.259
no	3	60	2	60.63	73.88	83.59	10.38
Arg	3	60	1	52.28	74.31	86.27	14.98
Arg	3	60	2	64.57	75.32	83.96	9.163
Lys	3	60	1	66.37	75.24	83.73	8.053
Lys	3	60	2	66.51	74.76	81.57	6.626
His	3	60	1	61.76	73.19	80.65	8.414
His	3	60	2	57.35	73.49	84.03	11.36
no	10	60	1	64.63	73.72	81.48	7.25
no	10	60	2	59.92	73.69	84.37	11.35
Arg	10	60	1	67.41	75.39	89.55	8.757
Arg	10	60	2	67.13	75.46	83.57	7.353
Lys	10	60	1	66.12	75.85	83.6	7.547
Lys	10	60	2	63.29	75.48	86.01	10.03
His	10	60	1	50.18	73.22	86.92	16.12
His	10	60	2	60.25	73.11	83.95	11.68

Oven-dried Beauregard DCS data

AA	pH	Time	Rep	Onset	Peak	End	Enthalpy
Control	no	0min	1	54.46	64.98	83.68	13.72
Control	no	0min	2	53.91	64.5	85.4	14.48
Arg	no	0min	1	53.82	65.89	83.7	14.33
Arg	no	0min	2	56.48	66.23	87.02	12.62
Lys	no	0min	1	55.86	66.4	86.46	14.53
Lys	no	0min	2	56.86	65.93	83.47	11.84
His	no	0min	1	55.78	65.31	84.85	13
His	no	0min	2	55.7	65.35	82.72	14.58
noaa	3	30min	1	59.64	71.18	84.33	10.86
noaa	3	30min	2	59.66	70.9	82.24	10.85
Arg	3	30min	1	61.04	72.69	83.12	11.24
Arg	3	30min	2	57.74	72.76	89.92	15.08
Lys	3	30min	1	55.26	71.83	84.69	15.93
Lys	3	30min	2	58.39	72.05	85.22	13.58
His	3	30min	1	57.78	70.42	83.01	14.01
His	3	30min	2	59.36	71.13	83.99	12.79
noaa	10	30min	1	58.17	71.09	82.69	13.72
noaa	10	30min	2	56.24	70.9	85.22	15.44
Arg	10	30min	1	58.17	72.45	82.91	13.82
Arg	10	30min	2	54.74	72.52	87.31	17.54
Lys	10	30min	1	54.7	72.01	86.1	16.15
Lys	10	30min	2	57.04	71.85	84.24	14.18
His	10	30min	1	57.94	71.06	82.54	13.46
His	10	30min	2	57.13	70.74	83.52	14.89

Oven-dried Evangeline DCS data

AA	pH	Time	Rep	Onset	Peak	End	Enthalpy
Control	no	0min	1	53.49	74.8	85.36	16.02
Control	no	0min	2	52.95	74.3	85.22	15.92
Arg	no	0min	1	54.54	76.17	86.65	15.3
Arg	no	0min	2	53.99	76.42	85.67	13.65
Lys	no	0min	1	54.45	75.83	88.44	15.68
Lys	no	0min	2	53.91	76.22	87.19	15.78
His	no	0min	1	55.02	74.74	85.66	14.3
His	no	0min	2	54.13	74.43	83.65	14.98
noaa	3	30min	1	57.49	73.72	85.08	13.6
noaa	3	30min	2	58.69	74.69	85.75	11.68
Arg	3	30min	1	58.09	75.24	84.11	11.53
Arg	3	30min	2	56.28	74.71	82.47	11.6
Lys	3	30min	1	57.12	74.94	86.72	14.85
Lys	3	30min	2	56	74.44	85.97	15.11

His	3	30min	1	54.9	73.58	87.01	15.45
His	3	30min	2	57.64	73.64	82.99	61.06
noaa	10	30min	1	59.5	74.31	83.3	10.52
noaa	10	30min	2	55.56	74.11	85.37	14.16
Arg	10	30min	1	55.93	74.95	87.54	15.05
Arg	10	30min	2	55.48	75.06	89.25	15.66
Lys	10	30min	1	56.94	74.71	87.44	14.71
Lys	10	30min	2	55.77	75.75	85.47	13.5
His	10	30min	1	55.11	73.66	86.79	15.21
His	10	30min	2	54.72	73.84	86.56	15.46

```
dm "clear log; clear output";
      options nodate nonumber;
      data FD DSC;
      input treat $ rep Onset Peak Conclusion Enthalpy;

      datalines;
1     1     57.73 73.96 83.97 9.233
1     2     56.53 74.3  84.26 8.635
2     1     65.31 75.8  86.39 5.436
2     2     68.23 75.12 83.34 3.104
;
      proc sort; by treat;
      run;
      proc means mean std n maxdec=2;by treat;
      var Onset Peak Conclusion Enthalpy;
      run;
      proc glm;
      class treat;
      model Onset Peak Conclusion Enthalpy =treat;
means treat / tukey lines;
run; quit;
```

APPENDIX 2: RVA RAW DATA AND SAS CODE

Freeze-dried RVA data

Type	AA	pH	Time	Rep	PV	MV	BD	FV	TSB	Tp	PT
b	noaa	no	0min	1	8005	2762	5243	3951	1189	3.87	72.35
b	noaa	no	0min	2	7860	2818	5042	3851	1033	4.07	72.35
b	water	no	0min	1	7830	2877	4953	3946	1069	4.2	71.55
b	water	no	0min	2	7817	2909	4908	3932	1023	4.2	71.65
b	Arg	no	0min	1	6278	1590	4688	3240	1650	4.27	74
b	Arg	no	0min	2	6296	1695	4601	3345	1650	4.27	73.95
b	Lys	no	0min	1	6079	2007	4072	3290	1283	4.33	74
b	Lys	no	0min	2	6302	2060	4242	3366	1306	4.27	74.05
b	His	no	0min	1	6622	2577	4045	3482	905	4.07	71.6
b	His	no	0min	2	6541	2590	3951	3449	859	4.07	71.5
b	noaa	3	30min	1	6767	2599	4168	3545	946	3.87	70.75
b	noaa	3	30min	2	6844	2578	4266	3473	895	3.93	70.75
b	Arg	3	30min	1	7489	2389	5100	3776	1387	4	73.25
b	Arg	3	30min	2	7506	2399	5107	3745	1346	4	73.2
b	Lys	3	30min	1	7412	2439	4973	3607	1168	4	73.05
b	Lys	3	30min	2	7142	2384	4758	3633	1249	4.07	73.2
b	His	3	30min	1	6995	2640	4355	3492	852	4	71.65
b	His	3	30min	2	7056	2654	4402	3466	812	4	71.55
b	noaa	10	30min	1	8513	2990	5523	4068	1078	3.93	70.85
b	noaa	10	30min	2	8137	2883	5254	3939	1056	4	70.8
b	Arg	10	30min	1	6941	2266	4675	3452	1186	4	73.2
b	Arg	10	30min	2	6884	2196	4688	3418	1222	4.07	73.2

Type	AA	pH	Time	Rep	PV	MV	BD	FV	TSB	Tp	PT
b	Lys	10	30min	1	7031	2334	4697	3530	1196	4.07	73.35
b	Lys	10	30min	2	6820	4512	3380	1072	4.07	4.07	73.15
b	His	10	30min	1	7058	2614	4444	3503	889	4	71.5
b	His	10	30min	2	6916	2669	4247	3491	822	4.07	71.5
b	noaa	3	1hour	1	5355	2046	3309	2870	824	3.87	71.6
b	noaa	3	1hour	2	5398	2036	3362	2830	794	3.87	71.65
b	Arg	3	1hour	1	6702	2168	4534	3390	1222	4.07	73.3
b	Arg	3	1hour	2	6341	2001	4340	3394	1393	4.07	74.05
b	Lys	3	1hour	1	6806	2377	4429	3502	1125	4.07	73.2
b	Lys	3	1hour	2	6746	2375	4371	3339	964	4.07	73.25
b	His	3	1hour	1	6821	2614	4207	3475	861	4	71.65
b	His	3	1hour	2	6792	2659	4133	3477	818	4	71.55
b	noaa	10	1hour	1	7228	2740	4488	3621	881	4	71.5
b	noaa	10	1hour	2	7237	2787	4450	3584	797	4	71.6
b	Arg	10	1hour	1	6457	2057	4400	3342	1285	4.13	74
b	Arg	10	1hour	2	6594	2089	4505	3319	1230	4.07	73.3
b	Lys	10	1hour	1	6689	2268	4421	3273	1005	4	73.2
b	Lys	10	1hour	2	6655	2265	4390	3312	1047	4.07	73.15
b	His	10	1hour	1	6666	2578	4088	3465	887	4	71.65
b	His	10	1hour	2	6678	2601	4077	3469	868	4.07	71.55
E	noaa	no	0min	1	7417	2880	4537	3821	941	4.27	74
E	noaa	no	0min	2	7499	2928	4571	3820	892	4.2	74.05
E	water	no	0min	1	7537	2933	4604	3773	840	4.2	71.6
E	water	no	0min	2	7524	2919	4605	3762	843	4.2	71.5

Type	AA	pH	Time	Rep	PV	MV	BD	FV	TSB	Tp	PT
E	Arg	no	0min	1	5520	1550	3970	3291	1741	4.4	74
E	Arg	no	0min	2	5977	1754	4223	3243	1489	4.33	74
E	Lys	no	0min	1	5457	1857	3600	3180	1323	4.4	73.95
E	Lys	no	0min	2	5348	1866	3482	3152	1286	4.4	73.95
E	His	no	0min	1	5822	2326	3496	3280	954	4.33	71.65
E	His	no	0min	2	6156	2412	3744	3459	1047	4.33	71.5
E	noaa	3	30min	1	7088	2700	4388	3612	912	4.07	73.15
E	noaa	3	30min	2	6977	2693	4284	3604	911	4.07	73.15
E	Arg	3	30min	1	6506	2221	4285	3519	1298	4.2	74.8
E	Arg	3	30min	2	7204	2505	4699	3686	1181	4.2	74.75
E	Lys	3	30min	1	7040	2745	4295	3778	1033	4.2	74.85
E	Lys	3	30min	2	7008	2699	4309	3813	1114	4.2	74.9
E	His	3	30min	1	7010	2880	4130	3739	859	4.13	73.25
E	His	3	30min	2	7245	2920	4325	3777	857	4.07	73.25
E	noaa	10	30min	1	7774	3015	4759	3840	825	4.07	73.25
E	noaa	10	30min	2	7789	2974	4815	3794	820	4.2	73.15
E	Arg	10	30min	1	7021	2452	4569	3689	1237	4.27	74.8
E	Arg	10	30min	2	7080	2548	4532	3547	999	4.2	74.75
E	Lys	10	30min	1	6879	2643	4236	3783	1140	4.2	74.8
E	Lys	10	30min	2	5717	2357	3360	3286	929	4.07	74.85
E	His	10	30min	1	7187	2855	4332	3710	855	4.07	73.3
E	His	10	30min	2	7083	2815	4268	3719	904	4.13	73.3
E	noaa	3	1hour	1	6854	2597	4257	3545	948	4.07	73.2
E	noaa	3	1hour	2	6861	2611	4250	3580	969	4.7	73.2

Type	AA	pH	Time	Rep	PV	MV	BD	FV	TSB	Tp	PT
E	Arg	3	1hour	1	6971	2432	4485	3442	1010	4.2	74.7
E	Arg	3	1hour	2	6980	2372	4608	3564	1192	4.2	75.55
E	Lys	3	1hour	1	6925	2628	4297	3763	1135	4.2	74.85
E	Lys	3	1hour	2	6968	2636	4332	3744	1108	4.2	74.8
E	His	3	1hour	1	7097	2904	4193	3758	854	4.2	73.2
E	His	3	1hour	2	7177	2892	4285	3781	889	4.2	73.15
E	noaa	10	1hour	1	7645	3013	4632	3859	846	4.2	73.2
E	noaa	10	1hour	2	7624	2984	4640	3875	891	4.2	73.15
E	Arg	10	1hour	1	6911	2366	4545	3517	1151	4.2	74.8
E	Arg	10	1hour	2	7061	2456	4605	3756	1300	4.2	74.85
E	Lys	10	1hour	1	6781	2615	4166	3663	1048	4.2	74.85
E	Lys	10	1hour	2	6824	2606	4218	3767	1161	4.2	74.8
E	His	10	1hour	1	7086	2867	4219	3729	862	4.2	73.15
E	His	10	1hour	2	7156	2889	4267	3723	834	4.2	73.3

Oven-dried RVA data

Type	AA	pH	Time	Rep	PV	MV	BD	FV	TSB	Tp	PT
B	Native	no	0min	1	8005	2762	5243	3951	1189	3.87	72.35
B	Native	no	0min	2	7860	2818	5042	3851	1033	4.07	72.35
B	Control	no	0min	1	5924	2364	3560	3281	917	4.2	69.25
B	Control	no	0min	2	5821	2317	3504	3248	931	4.2	69.15
B	Arg	no	0min	1	5371	1557	3814	3176	1619	4.27	70.8
B	Arg	no	0min	2	5430	1530	3900	3188	1658	4.27	70.75
B	Lys	no	0min	1	5246	1644	3602	3005	1361	4.27	70.75
B	Lys	no	0min	2	5278	1554	3724	2956	1402	4.27	69.9
B	His	no	0min	1	5064	2108	2956	2947	839	4.2	69.05
B	His	no	0min	2	4966	2031	2935	2894	863	4.2	69.9
B	Noaa	3	30min	1	3782	1868	1914	2703	835	4.13	73.25
B	Noaa	3	30min	2	3799	1846	1953	2671	825	4.13	73.25
B	Arg	3	30min	1	2997	1427	1570	2108	681	4.27	74.85
B	Arg	3	30min	2	2998	1484	1514	2191	707	4.2	74.85
B	Lys	3	30min	1	2943	1476	1467	2129	653	4.27	74.75
B	Lys	3	30min	2	2939	1484	1455	2146	662	4.27	74.05
B	His	3	30min	1	3207	1722	1485	2439	717	4.2	74.05
B	His	3	30min	2	3263	1742	1521	2532	790	4.2	73.2
B	Noaa	10	30min	1	4145	1951	2194	2820	869	4.13	73.15
B	Noaa	10	30min	2	4172	1941	2231	2827	886	4.13	73.95
B	Arg	10	30min	1	3241	1495	1746	2198	703	4.27	74.75
B	Arg	10	30min	2	3275	1554	1721	2301	747	4.2	74.8
B	Lys	10	30min	1	2931	1449	1482	2114	665	4.27	74.8
B	Lys	10	30min	2	2915	1457	1458	2113	656	4.27	74.05
B	His	10	30min	1	3602	1866	1736	2715	849	4.2	73.2
B	His	10	30min	2	3680	1894	1786	2747	853	4.2	73.2
E	Native	no	0min	1	7417	2880	4537	3821	941	4.27	74
E	Native	no	0min	2	7499	2928	4571	3820	892	4.2	74.05
E	Control	no	0min	1	5971	2658	3313	3440	782	4.33	74
E	Control	no	0min	2	6153	2664	3489	3510	846	4.27	73.25
E	Arg	no	0min	1	5079	1351	3728	3130	1779	4.33	74.85
E	Arg	no	0min	2	5201	1702	3499	3197	1495	4.4	74.8
E	Lys	no	0min	1	5094	1700	3394	3084	1384	4.4	74.75
E	Lys	no	0min	2	4979	1551	3428	3036	1485	4.4	74.8
E	His	no	0min	1	5633	2552	3081	3445	893	4.33	74
E	His	no	0min	2	5729	2566	3163	3467	901	4.33	73.95
E	noaa	3	30min	1	5715	2510	3205	3377	867	4.2	74.85
E	noaa	3	30min	2	5705	2493	3213	3390	897	4.2	74.1
E	Arg	3	30min	1	5081	1867	3214	3289	1422	4.27	75.55
E	Arg	3	30min	2	5167	1933	3234	3204	1271	4.27	75.55

Type	AA	pH	Time	Rep	PV	MV	BD	FV	TSB	Tp	PT
E	Lys	3	30min	1	5271	2078	3193	3325	1247	4.27	75.55
E	Lys	3	30min	2	5277	1995	3282	3284	1289	4.27	75.6
E	His	3	30min	1	5687	2524	3163	3450	926	4.2	74.8
E	His	3	30min	2	5678	2515	3163	3456	941	4.2	74
E	noaa	10	30min	1	5827	2545	3282	3487	942	4.2	74.85
E	noaa	10	30min	2	5826	2564	3262	3465	901	4.2	74.9
E	Arg	10	30min	1	5305	1987	3318	3283	1296	4.27	75.55
E	Arg	10	30min	2	5307	1950	3357	3318	1368	4.27	75.6
E	Lys	10	30min	1	4997	1930	3067	3175	1245	4.33	75.55
E	Lys	10	30min	2	5145	1957	3188	3192	1235	4.27	75.65
E	His	10	30min	1	5452	2458	2994	3393	935	4.2	74.05
E	His	10	30min	2	5452	2458	2994	3393	935	4.2	74.05

```
dm "clear log; clear output";
        options nodate nonumber;
        data oven-dried RVA samples;
        input treat $ rep PV MV BD FV TSB TP Pt;
        datalines;
        1       1       8005    2762    5243    3951    1189    3.87    72.35
        1       2       7860    2818    5042    3851    1033    4.07    72.35
        2       1       5924    2364    3560    3281    917     4.2     69.25
        2       2       5821    2317    3504    3248    931     4.2     69.15
        3       1       5371    1557    3814    3176    1619    4.27    70.8
        3       2       5430    1530    3900    3188    1658    4.27    70.75
        4       1       5246    1644    3602    3005    1361    4.27    70.75
        4       2       5278    1554    3724    2956    1402    4.27    69.9
        5       1       5064    2108    2956    2947    839     4.2     69.05
        5       2       4966    2031    2935    2894    863     4.2     69.9
        6       1       3782    1868    1914    2703    835     4.13    73.25
        6       2       3799    1846    1953    2671    825     4.13    73.25
        7       1       2997    1427    1570    2108    681     4.27    74.85
        7       2       2998    1484    1514    2191    707     4.2     74.85
        8       1       2943    1476    1467    2129    653     4.27    74.75
        8       2       2939    1484    1455    2146    662     4.27    74.05
        9       1       3207    1722    1485    2439    717     4.2     74.05
        9       2       3263    1742    1521    2532    790     4.2     73.2
        10      1       4145    1951    2194    2820    869     4.13    73.15
        10      2       4172    1941    2231    2827    886     4.13    73.95
        11      1       3241    1495    1746    2198    703     4.27    74.75
        11      2       3275    1554    1721    2301    747     4.2     74.8
        12      1       2931    1449    1482    2114    665     4.27    74.8
        12      2       2915    1457    1458    2113    656     4.27    74.05
        13      1       3602    1866    1736    2715    849     4.2     73.2
```

13	2	3680	1894	1786	2747	853	4.2	73.2
22	1	7417	2880	4537	3821	941	4.27	74
22	2	7499	2928	4571	3820	892	4.2	74.05
23	1	5971	2658	3313	3440	782	4.33	74
23	2	6153	2664	3489	3510	846	4.27	73.25
24	1	5079	1351	3728	3130	1779	4.33	74.85
24	2	5201	1702	3499	3197	1495	4.4	74.8
25	1	5094	1700	3394	3084	1384	4.4	74.75
25	2	4979	1551	3428	3036	1485	4.4	74.8
26	1	5633	2552	3081	3445	893	4.33	74
26	2	5729	2566	3163	3467	901	4.33	73.95
27	1	5715	2510	3205	3377	867	4.2	74.85
27	2	5705	2493	3213	3390	897	4.2	74.1
28	1	5081	1867	3214	3289	1422	4.27	75.55
28	2	5167	1933	3234	3204	1271	4.27	75.55
29	1	5271	2078	3193	3325	1247	4.27	75.55
29	2	5277	1995	3282	3284	1289	4.27	75.6
30	1	5687	2524	3163	3450	926	4.2	74.8
30	2	5678	2515	3163	3456	941	4.2	74
31	1	5827	2545	3282	3487	942	4.2	74.85
31	2	5826	2564	3262	3465	901	4.2	74.9
32	1	5305	1987	3318	3283	1296	4.27	75.55
32	2	5307	1950	3357	3318	1368	4.27	75.6
33	1	4997	1930	3067	3175	1245	4.33	75.55
33	2	5145	1957	3188	3192	1235	4.27	75.65
34	1	5452	2458	2994	3393	935	4.2	74.05
34	2	5452	2458	2994	3393	935	4.2	74.05

;

```
        proc sort; by treat;
        run;
        proc means mean std n maxdec=2;by treat;
        var PV MV BD FV TSB TP Pt;
        run;
        proc glm;
        class treat;
        model PV MV BD FV TSB TP Pt =treat;
means treat / tukey lines;
run; quit;
```

APPENDIX 3: RS RAW DATA AND SAS CODE

Freeze-dried RS results

Treatment	Type	AA	pH	Time	Rep	RS% (A)	RS% (B)
1	b	Native	no	0min	1	10.28	10.91
1	b	Native	no	0min	2	10.61	10.39
2	b	Control	no	0min	1	13.41	12.46
2	b	control	no	0min	2	10.69	10.03
3	b	Arg	no	0min	1	11.27	14.26
3	b	Arg	no	0min	2	14.41	12.73
4	b	Lys	no	0min	1	15.1	14.14
4	b	Lys	no	0min	2	11.86	13.32
5	b	His	no	0min	1	12.01	13.01
5	b	His	no	0min	2	11.35	11.98
6	b	noaa	3	30min	1	9.22	9.38
6	b	noaa	3	30min	2	9.44	9.74
7	b	Arg	3	30min	1	11.04	11.29
7	b	Arg	3	30min	2	10.84	10.71
8	b	Lys	3	30min	1	11.93	11.63
8	b	Lys	3	30min	2	11.34	12.01
9	b	His	3	30min	1	11.1	10.79
9	b	His	3	30min	2	10.42	10.46
10	b	noaa	10	30min	1	10.73	10.49
10	b	noaa	10	30min	2	10.02	7.36
11	b	Arg	10	30min	1	11	11.4
11	b	Arg	10	30min	2	11.11	11.68
12	b	Lys	10	30min	1	10.19	10.2
12	b	Lys	10	30min	2	11.86	11.18
13	b	His	10	30min	1	9.5	10.42
13	b	His	10	30min	2	10.51	10.92
14	b	noaa	3	1hour	1	10.62	9.48
14	b	noaa	3	1hour	2	9.65	9.32
15	b	Arg	3	1hour	1	12.54	11.19
15	b	Arg	3	1hour	2	11.34	10.69
16	b	Lys	3	1hour	1	11.16	14.41
16	b	Lys	3	1hour	2	11.83	11.8
17	b	His	3	1hour	1	13.12	11.97
17	b	His	3	1hour	2	10.6	10.92
18	b	noaa	10	1hour	1	8.28	9.1
18	b	noaa	10	1hour	2	8.79	9.37
19	b	Arg	10	1hour	1	13.11	12.87
19	b	Arg	10	1hour	2	10.74	10.03

Treatment	Type	AA	pH	Time	Rep	RS% (A)	RS% (B)
20	b	Lys	10	1hour	1	12.99	12.62
20	b	Lys	10	1hour	2	10.09	10.56
21	b	His	10	1hour	1	12.39	13.36
21	b	His	10	1hour	2	10.8	10.7
22	e	noaa	no	0min	1	12.11	12.33
22	e	noaa	no	0min	2	12.28	12.16
23	e	water	no	0min	1	13.12	12.58
23	e	water	no	0min	2	13.59	13.67
24	e	Arg	no	0min	1	13.14	13.98
24	e	Arg	no	0min	2	16.8	12.55
25	e	Lys	no	0min	1	13.79	13.28
25	e	Lys	no	0min	2	13.96	13.89
26	e	His	no	0min	1	12.7	13
26	e	His	no	0min	2	13.37	12.55
27	e	noaa	3	30min	1	11.71	10.16
27	e	noaa	3	30min	2	11.96	12.15
28	e	Arg	3	30min	1	13.36	12.21
28	e	Arg	3	30min	2	13	13.31
29	e	Lys	3	30min	1	11.04	11.89
29	e	Lys	3	30min	2	12.67	13.09
30	e	His	3	30min	1	11.46	13.16
30	e	His	3	30min	2	12.61	13.14
31	e	noaa	10	30min	1	15.11	13.49
31	e	noaa	10	30min	2	14.08	13.91
32	e	Arg	10	30min	1	12.55	12.71
32	e	Arg	10	30min	2	13.15	12.09
33	e	Lys	10	30min	1	12.28	11.69
33	e	Lys	10	30min	2	11.65	12.13
34	e	His	10	30min	1	12.11	12.75
34	e	His	10	30min	2	12.64	12.6
35	e	noaa	3	1hour	1	12.49	13.41
35	e	noaa	3	1hour	2	12.46	11.78
36	e	Arg	3	1hour	1	14.14	13.42
36	e	Arg	3	1hour	2	14.14	14.53
37	e	Lys	3	1hour	1	12.03	12.38
37	e	Lys	3	1hour	2	12.6	11.9
38	e	His	3	1hour	1	13.05	12.65
38	e	His	3	1hour	2	15.36	13.91
39	e	noaa	10	1hour	1	12.62	12.99
39	e	noaa	10	1hour	2	14.14	13.13
40	e	Arg	10	1hour	1	13.62	13.34

Treatment	Type	AA	pH	Time	Rep	RS% (A)	RS% (B)
40	e	Arg	10	1hour	2	14.25	13.06
41	e	Lys	10	1hour	1	12.86	13.6
41	e	Lys	10	1hour	2	13.61	14.5
42	e	His	10	1hour	1	13.25	13.79
42	e	His	10	1hour	2	13.28	13.49

Oven-dried RS results

Treatment	Type	AA	pH	Rep	RS% (A)	RS% (B)
B	1	Native	no	1	10.28	10.91
B	1	Native	no	2	10.14	10.58
B	2	Control	no	1	10.4	10.93
B	2	Control	no	2	10.88	11.34
B	3	Arg	no	1	11.42	12.4
B	3	Arg	no	2	11.87	12.75
B	4	Lys	no	1	12.44	11.61
B	4	Lys	no	2	12.35	11.87
B	5	His	no	1	10.41	11.43
B	5	His	no	2	11.75	11.85
B	6	noaa	3	1	15.61	15.29
B	6	noaa	3	2	14.36	15.2
B	7	Arg	3	1	13.02	12.7
B	7	Arg	3	2	12.45	11.23
B	8	Lys	3	1	16.14	14.56
B	8	Lys	3	2	15.85	16.44
B	9	His	3	1	16.51	15.97
B	9	His	3	2	14.68	15.91
B	10	noaa	10	1	15.7	16.14
B	10	noaa	10	2	16.74	16.97
B	11	Arg	10	1	16.35	16.96
B	11	Arg	10	2	16.76	16.92
B	12	Lys	10	1	16.02	17.29
B	12	Lys	10	2	15.49	15.57
B	13	His	10	1	15.72	16.47
B	13	His	10	2	16.77	16.62
E	22	Native	no	1	12.96	12.37
E	22	Native	no	2	12.11	12.33
E	23	Control	no	1	13.05	13.05
E	23	Control	no	2	13.3	13.87
E	24	Arg	no	1	15.42	15.78

Treatment	Type	AA	pH	Rep	RS% (A)	RS% (B)
E	24	Arg	no	2	15.1	15.63
E	25	Lys	no	1	14.03	14.16
E	25	Lys	no	2	12.56	13.5
E	26	His	no	1	15.04	15.42
E	26	His	no	2	14.26	14.51
E	27	noaa	3	1	15.92	16.01
E	27	noaa	3	2	16.13	15.64
E	28	Arg	3	1	15.07	15.82
E	28	Arg	3	2	15.78	17.06
E	29	Lys	3	1	14.04	16.32
E	29	Lys	3	2	14.31	14.94
E	30	His	3	1	15.63	15.93
E	30	His	3	2	14.44	14.26
E	31	noaa	10	1	18.43	17.53
E	31	noaa	10	2	18.86	18.63
E	32	Arg	10	1	11.59	12.8
E	32	Arg	10	2	12.37	11.77
E	33	Lys	10	1	19.73	18.11
E	33	Lys	10	2	19.86	19.78
E	34	His	10	1	19.05	18.11
E	34	His	10	2	18.54	18.6

```
dm "clear log; clear output";
        options nodate nonumber;
        data RS;
        input treat $ aa $ ph $ time $ Evangeline;
        datalines;
1       Native no       0min    12.11
1       Native no       0min    12.28
1       Native no       0min    12.33
1       Native no       0min    12.16
2       Control         no      0min    13.12
2       Control         no      0min    13.59
2       Control         no      0min    12.58
2       Control         no      0min    13.67
3       Arg     no      0min    13.14
3       Arg     no      0min    16.8
3       Arg     no      0min    13.98
3       Arg     no      0min    12.55
4       Lys     no      0min    13.79
4       Lys     no      0min    13.96
4       Lys     no      0min    13.28
4       Lys     no      0min    13.89
5       His     no      0min    12.7
```

5	His	no	0min	13.37
5	His	no	0min	13
5	His	no	0min	12.55
6	noaa	3	30min	11.71
6	noaa	3	30min	11.96
6	noaa	3	30min	10.16
6	noaa	3	30min	12.15
7	Arg	3	30min	13.36
7	Arg	3	30min	13
7	Arg	3	30min	12.21
7	Arg	3	30min	13.31
8	Lys	3	30min	11.04
8	Lys	3	30min	12.67
8	Lys	3	30min	11.89
8	Lys	3	30min	13.09
9	His	3	30min	11.46
9	His	3	30min	12.61
9	His	3	30min	13.16
9	His	3	30min	13.14
10	noaa	10	30min	15.11
10	noaa	10	30min	14.08
10	noaa	10	30min	13.49
10	noaa	10	30min	13.91
11	Arg	10	30min	12.55
11	Arg	10	30min	13.15
11	Arg	10	30min	12.71
11	Arg	10	30min	12.09
12	Lys	10	30min	12.28
12	Lys	10	30min	11.65
12	Lys	10	30min	11.69
12	Lys	10	30min	12.13
13	His	10	30min	12.11
13	His	10	30min	12.64
13	His	10	30min	12.75
13	His	10	30min	12.6
14	noaa	3	1hour	12.49
14	noaa	3	1hour	12.46
14	noaa	3	1hour	13.41
14	noaa	3	1hour	11.78
15	Arg	3	1hour	14.14
15	Arg	3	1hour	14.14
15	Arg	3	1hour	13.42
15	Arg	3	1hour	14.53
16	Lys	3	1hour	12.03
16	Lys	3	1hour	12.6
16	Lys	3	1hour	12.38
16	Lys	3	1hour	11.9
17	His	3	1hour	13.05
17	His	3	1hour	15.36

```
17      His     3       1hour   12.65
17      His     3       1hour   13.91
18      noaa    10      1hour   12.62
18      noaa    10      1hour   14.14
18      noaa    10      1hour   12.99
18      noaa    10      1hour   13.13
19      Arg     10      1hour   13.62
19      Arg     10      1hour   14.25
19      Arg     10      1hour   13.34
19      Arg     10      1hour   13.06
20      Lys     10      1hour   12.86
20      Lys     10      1hour   13.61
20      Lys     10      1hour   13.6
20      Lys     10      1hour   14.5
21      His     10      1hour   13.25
21      His     10      1hour   13.28
21      His     10      1hour   13.79
21      His     10      1hour   13.49

;
        proc sort; by treat;
        run;
        proc means mean std n maxdec=2;by treat;
        var Evangeline;
        run;
        proc glm;
        class treat;
        model Evangeline=treat;
means treat / tukey lines;


run; quit;


dm "clear log; clear output";

        options nodate nonumber;
        data Oven-Dried RS;
        input treat $ aa $ ph $ Beauregard Evangeline;
        datalines;


;
        proc sort; by treat;
        run;
        proc means mean std n maxdec=2;by treat;
        var Beauregard Evangeline;
        run;
        proc glm;
        class treat;
```

```
        model Beauregard Evangeline=treat;
means treat / tukey lines;


run; quit;
```

t-test

```
dm 'log;clear;output;clear';
data one;
input treat $ type$ aa $ pH $ time $ rep A B;
datalines;
```

1	b	Native	no	0min	1	10.28	10.91
1	b	Native	no	0min	2	10.61	10.39
2	b	Control	no	0min	1	13.41	12.46
2	b	control	no	0min	2	10.69	10.03
3	b	Arg	no	0min	1	11.27	14.26
3	b	Arg	no	0min	2	14.41	12.73
4	b	Lys	no	0min	1	15.1	14.14
4	b	Lys	no	0min	2	11.86	13.32
5	b	His	no	0min	1	12.01	13.01
5	b	His	no	0min	2	11.35	11.98
6	b	noaa	3	30min	1	9.22	9.38
6	b	noaa	3	30min	2	9.44	9.74
7	b	Arg	3	30min	1	11.04	11.29
7	b	Arg	3	30min	2	10.84	10.71
8	b	Lys	3	30min	1	11.93	11.63
8	b	Lys	3	30min	2	11.34	12.01
9	b	His	3	30min	1	11.1	10.79
9	b	His	3	30min	2	10.42	10.46
10	b	noaa	10	30min	1	10.73	10.49
10	b	noaa	10	30min	2	10.02	7.36
11	b	Arg	10	30min	1	11	11.4
11	b	Arg	10	30min	2	11.11	11.68
12	b	Lys	10	30min	1	10.19	10.2
12	b	Lys	10	30min	2	11.86	11.18
13	b	His	10	30min	1	9.5	10.42
13	b	His	10	30min	2	10.51	10.92
14	b	noaa	3	1hour	1	10.62	9.48
14	b	noaa	3	1hour	2	9.65	9.32
15	b	Arg	3	1hour	1	12.54	11.19
15	b	Arg	3	1hour	2	11.34	10.69
16	b	Lys	3	1hour	1	11.16	14.41
16	b	Lys	3	1hour	2	11.83	11.8
17	b	His	3	1hour	1	13.12	11.97
17	b	His	3	1hour	2	10.6	10.92

18	b	noaa		10	1hour	1	8.28	9.1
18	b	noaa		10	1hour	2	8.79	9.37
19	b	Arg		10	1hour	1	13.11	12.87
19	b	Arg		10	1hour	2	10.74	10.03
20	b	Lys		10	1hour	1	12.99	12.62
20	b	Lys		10	1hour	2	10.09	10.56
21	b	His		10	1hour	1	12.39	13.36
21	b	His		10	1hour	2	10.8	10.7
22	e	noaa	no		0min	1	12.11	12.33
22	e	noaa	no		0min	2	12.28	12.16
23	e	water	no		0min	1	13.12	12.58
23	e	water	no		0min	2	13.59	13.67
24	e	Arg	no		0min	1	13.14	13.98
24	e	Arg	no		0min	2	16.8	12.55
25	e	Lys	no		0min	1	13.79	13.28
25	e	Lys	no		0min	2	13.96	13.89
26	e	His	no		0min	1	12.7	13
26	e	His	no		0min	2	13.37	12.55
27	e	noaa		3	30min	1	11.71	10.16
27	e	noaa		3	30min	2	11.96	12.15
28	e	Arg		3	30min	1	13.36	12.21
28	e	Arg		3	30min	2	13	13.31
29	e	Lys		3	30min	1	11.04	11.89
29	e	Lys		3	30min	2	12.67	13.09
30	e	His		3	30min	1	11.46	13.16
30	e	His		3	30min	2	12.61	13.14
31	e	noaa		10	30min	1	15.11	13.49
31	e	noaa		10	30min	2	14.08	13.91
32	e	Arg		10	30min	1	12.55	12.71
32	e	Arg		10	30min	2	13.15	12.09
33	e	Lys		10	30min	1	12.28	11.69
33	e	Lys		10	30min	2	11.65	12.13
34	e	His		10	30min	1	12.11	12.75
34	e	His		10	30min	2	12.64	12.6
35	e	noaa		3	1hour	1	12.49	13.41
35	e	noaa		3	1hour	2	12.46	11.78
36	e	Arg		3	1hour	1	14.14	13.42
36	e	Arg		3	1hour	2	14.14	14.53
37	e	Lys		3	1hour	1	12.03	12.38
37	e	Lys		3	1hour	2	12.6	11.9
38	e	His		3	1hour	1	13.05	12.65
38	e	His		3	1hour	2	15.36	13.91
39	e	noaa		10	1hour	1	12.62	12.99
39	e	noaa		10	1hour	2	14.14	13.13
40	e	Arg		10	1hour	1	13.62	13.34

```
        40   e        Arg        10   1hour        2    14.25   13.06
        41   e        Lys        10   1hour        1    12.86    13.6
        41   e        Lys        10   1hour        2    13.61    14.5
        42   e        His        10   1hour        1    13.25   13.79
        42   e        His        10   1hour        2    13.28   13.49
;
proc ttest;
class type;
var A--B;
```

APPENDIX 4: SAS RAW DATA AND SAS CODE

Freeze-dried SDS data

AA	pH	Time	SDS % (B)	SDS % (E)
Native	no	0min	6.57	2.44
Native	no	0min	6.5	2.3
Native	no	0min	5.28	2.63
Native	no	0min	4.51	2.44
Control	no	0min	9.21	0.24
Control	no	0min	9.36	0.36
Control	no	0min	9.49	0.5
Control	no	0min	9.42	0.47
Arg	no	0min	13.1	1.13
Arg	no	0min	14.26	1.24
Arg	no	0min	13.64	1.1
Arg	no	0min	13.5	1.18
Lys	no	0min	3.28	1.12
Lys	no	0min	3.94	1.18
Lys	no	0min	3.87	1.84
Lys	no	0min	4.27	1.18
His	no	0min	4.36	5.95
His	no	0min	5.83	5.48
His	no	0min	3.86	6.8
His	no	0min	3.14	6.68
noaa	3	30min	7.5	1.46
noaa	3	30min	7.34	1.62
noaa	3	30min	7.65	1.62
noaa	3	30min	7.84	1.52
Arg	3	30min	11.82	0.76
Arg	3	30min	11.45	0.75
Arg	3	30min	11.35	0.83
Arg	3	30min	11.14	0.71
Lys	3	30min	11.82	7.99
Lys	3	30min	12.16	8.28
Lys	3	30min	12.09	8.24
Lys	3	30min	11.88	8.08
His	3	30min	3.96	11.76
His	3	30min	4.61	12.21
His	3	30min	4.29	12.03
His	3	30min	3.8	11.13
noaa	10	30min	11.52	5.09
noaa	10	30min	11.35	5.28

AA	pH	Time	SDS % (B)	SDS % (E)
noaa	10	30min	12.23	5.75
noaa	10	30min	11.36	5.2
Arg	10	30min	4.73	6.31
Arg	10	30min	3.95	6.6
Arg	10	30min	4.17	7.64
Arg	10	30min	4.18	6.69
Lys	10	30min	6.8	2.32
Lys	10	30min	6.54	2.46
Lys	10	30min	6.66	2.35
Lys	10	30min	6.39	2.1
His	10	30min	15.31	5.45
His	10	30min	15.15	5.87
His	10	30min	15.05	5.78
His	10	30min	15.56	5.42
noaa	3	1hour	15.18	2.58
noaa	3	1hour	14.64	2.59
noaa	3	1hour	14.21	2.77
noaa	3	1hour	15.4	2.82
Arg	3	1hour	2.17	0.65
Arg	3	1hour	2.15	0.34
Arg	3	1hour	2.01	0.16
Arg	3	1hour	2.21	0.13
Lys	3	1hour	13.4	5.29
Lys	3	1hour	14.3	5.7
Lys	3	1hour	13.83	5.88
Lys	3	1hour	13.55	5.7
His	3	1hour	14.6	6
His	3	1hour	14.1	6.76
His	3	1hour	15.16	6.08
His	3	1hour	14.85	6.2
noaa	10	1hour	1.52	10.54
noaa	10	1hour	1.43	10.45
noaa	10	1hour	2.01	10.72
noaa	10	1hour	2.1	10.11
Arg	10	1hour	1.14	4.66
Arg	10	1hour	1.16	4.06
Arg	10	1hour	1.1	4.37
Arg	10	1hour	1.87	4.85
Lys	10	1hour	2.43	3.43
Lys	10	1hour	2.29	4.28
Lys	10	1hour	2.89	4.16

AA	pH	Time	SDS % (B)	SDS % (E)
Lys	10	1hour	2.56	3.76
His	10	1hour	7.39	6.63
His	10	1hour	7.15	6.59
His	10	1hour	7.89	5.62
His	10	1hour	7.63	6.87

Oven-dried SDS data

AA	pH	SDS % (B)	SDS % (E)
Native	no	6.57	2.44
Native	no	6.5	2.3
Native	no	5.28	2.63
Native	no	4.51	2.44
Control	no	8.14	10.1
Control	no	7.94	9.9
Control	no	8.62	10.05
Control	no	7.96	10.66
Arg	no	11.82	2.55
Arg	no	11.83	2.96
Arg	no	11.52	2.88
Arg	no	11.8	2.55
Lys	no	1.67	2.62
Lys	no	1.48	2.58
Lys	no	2.52	2.12
Lys	no	1.93	2.06
His	no	5.33	3.52
His	no	5.64	3.39
His	no	5.26	3.43
His	no	5.34	3.74
no	pH3	9.74	1.24
no	pH3	9.99	1.62
no	pH3	10.21	1.58
no	pH3	10.44	1.53
Arg	pH3	15.56	1.42
Arg	pH3	14.69	1.5
Arg	pH3	15.83	1.48
Arg	pH3	15.64	2.06
Lys	pH3	9.89	10.34
Lys	pH3	9.25	10.27
Lys	pH3	9.93	10.51

AA	pH	SDS % (B)	SDS % (E)
Lys	pH3	9.52	10.26
His	pH3	5.09	6.32
His	pH3	5.09	6.52
His	pH3	5.88	6.17
His	pH3	5.58	5.66
no	pH10	10.82	9.38
no	pH10	10.73	9.1
no	pH10	10.94	9.15
no	pH10	10.95	8.99
Arg	pH10	6.53	5.03
Arg	pH10	6.61	5.09
Arg	pH10	7.14	5.9
Arg	pH10	7.96	6.16
Lys	pH10	5.58	4.32
Lys	pH10	5.89	4.31
Lys	pH10	6.68	3.55
Lys	pH10	6.42	3.64
His	pH10	1.26	5.66
His	pH10	0.65	6.07
His	pH10	1.05	5.63
His	pH10	0.88	6.06

```
dm "clear log; clear output";
        options nodate nonumber;
        data FD SDS;
        input treat $ aa $ ph $ time $ Beauregard Evangeline;
        datalines;
    1       Native      no      0min    6.57    2.44
    1       Native      no      0min    6.5     2.3
    1       Native      no      0min    5.28    2.63
    1       Native      no      0min    4.51    2.44
    2       Control     no      0min    9.21    0.24
    2       Control     no      0min    9.36    0.36
    2       Control     no      0min    9.49    0.5
    2       Control     no      0min    9.42    0.47
    3       Arg         no      0min    13.1    1.13
    3       Arg         no      0min    14.26   1.24
    3       Arg         no      0min    13.64   1.1
    3       Arg         no      0min    13.5    1.18
    4       Lys         no      0min    3.28    1.12
    4       Lys         no      0min    3.94    1.18
```

4	Lys	no	0min	3.87	1.84
4	Lys	no	0min	4.27	1.18
5	His	no	0min	4.36	5.95
5	His	no	0min	5.83	5.48
5	His	no	0min	3.86	6.8
5	His	no	0min	3.14	6.68
6	noaa	3	30min	7.5	1.46
6	noaa	3	30min	7.34	1.62
6	noaa	3	30min	7.65	1.62
6	noaa	3	30min	7.84	1.52
7	Arg	3	30min	11.82	0.76
7	Arg	3	30min	11.45	0.75
7	Arg	3	30min	11.35	0.83
7	Arg	3	30min	11.14	0.71
8	Lys	3	30min	11.82	7.99
8	Lys	3	30min	12.16	8.28
8	Lys	3	30min	12.09	8.24
8	Lys	3	30min	11.88	8.08
9	His	3	30min	3.96	11.76
9	His	3	30min	4.61	12.21
9	His	3	30min	4.29	12.03
9	His	3	30min	3.8	11.13
10	noaa	10	30min	11.52	5.09
10	noaa	10	30min	11.35	5.28
10	noaa	10	30min	12.23	5.75
10	noaa	10	30min	11.36	5.2
11	Arg	10	30min	4.73	6.31
11	Arg	10	30min	3.95	6.6
11	Arg	10	30min	4.17	7.64
11	Arg	10	30min	4.18	6.69
12	Lys	10	30min	6.8	2.32
12	Lys	10	30min	6.54	2.46
12	Lys	10	30min	6.66	2.35
12	Lys	10	30min	6.39	2.1
13	His	10	30min	15.31	5.45
13	His	10	30min	15.15	5.87
13	His	10	30min	15.05	5.78
13	His	10	30min	15.56	5.42
14	noaa	3	1hour	15.18	2.58
14	noaa	3	1hour	14.64	2.59
14	noaa	3	1hour	14.21	2.77
14	noaa	3	1hour	15.4	2.82
15	Arg	3	1hour	2.17	0.65
15	Arg	3	1hour	2.15	0.34
15	Arg	3	1hour	2.01	0.16

```
15      Arg     3       1hour   2.21    0.13
16      Lys     3       1hour   13.4    5.29
16      Lys     3       1hour   14.3    5.7
16      Lys     3       1hour   13.83   5.88
16      Lys     3       1hour   13.55   5.7
17      His     3       1hour   14.6    6
17      His     3       1hour   14.1    6.76
17      His     3       1hour   15.16   6.08
17      His     3       1hour   14.85   6.2
18      noaa    10      1hour   1.52    10.54
18      noaa    10      1hour   1.43    10.45
18      noaa    10      1hour   2.01    10.72
18      noaa    10      1hour   2.1     10.11
19      Arg     10      1hour   1.14    4.66
19      Arg     10      1hour   1.16    4.06
19      Arg     10      1hour   1.1     4.37
19      Arg     10      1hour   1.87    4.85
20      Lys     10      1hour   2.43    3.43
20      Lys     10      1hour   2.29    4.28
20      Lys     10      1hour   2.89    4.16
20      Lys     10      1hour   2.56    3.76
21      His     10      1hour   7.39    6.63
21      His     10      1hour   7.15    6.59
21      His     10      1hour   7.89    5.62
21      His     10      1hour   7.63    6.87

;
        proc sort; by treat;
        run;
        proc means mean std n maxdec=2;by treat;
        var Evangeline;
        run;
        proc glm;
        class treat;
        model Evangeline=treat;
means treat / tukey lines;


dm "clear log; clear output";
        options nodate nonumber;
        data Oven-Dried SDS;
        input treat $ aa $ ph $ Beauregard Evangeline;
        datalines;

    1       Native      no      6.57    2.44
    1       Native      no       6.5     2.3
    1       Native      no      5.28    2.63
```

1	Native	no	4.51	2.44
2	Control	no	8.14	10.1
2	Control	no	7.94	9.9
2	Control	no	8.62	10.05
2	Control	no	7.96	10.66
3	Arg	no	11.82	2.55
3	Arg	no	11.83	2.96
3	Arg	no	11.52	2.88
3	Arg	no	11.8	2.55
4	Lys	no	1.67	2.62
4	Lys	no	1.48	2.58
4	Lys	no	2.52	2.12
4	Lys	no	1.93	2.06
5	His	no	5.33	3.52
5	His	no	5.64	3.39
5	His	no	5.26	3.43
5	His	no	5.34	3.74
6	no	pH3	9.74	1.24
6	no	pH3	9.99	1.62
6	no	pH3	10.21	1.58
6	no	pH3	10.44	1.53
7	Arg	pH3	15.56	1.42
7	Arg	pH3	14.69	1.5
7	Arg	pH3	15.83	1.48
7	Arg	pH3	15.64	2.06
8	Lys	pH3	9.89	10.34
8	Lys	pH3	9.25	10.27
8	Lys	pH3	9.93	10.51
8	Lys	pH3	9.52	10.26
9	His	pH3	5.09	6.32
9	His	pH3	5.09	6.52
9	His	pH3	5.88	6.17
9	His	pH3	5.58	5.66
10	no	pH10	10.82	9.38
10	no	pH10	10.73	9.1
10	no	pH10	10.94	9.15
10	no	pH10	10.95	8.99
11	Arg	pH10	6.53	5.03
11	Arg	pH10	6.61	5.09
11	Arg	pH10	7.14	5.9
11	Arg	pH10	7.96	6.16
12	Lys	pH10	5.58	4.32
12	Lys	pH10	5.89	4.31
12	Lys	pH10	6.68	3.55
12	Lys	pH10	6.42	3.64

```
    13          His         pH10        1.26        5.66
    13          His         pH10        0.65        6.07
    13          His         pH10        1.05        5.63
    13          His         pH10        0.88        6.06
;
          proc sort; by treat;
          run;
          proc means mean std n maxdec=2;by treat;
          var Evangeline;
          run;
          proc glm;
          class treat;
          model Evangeline=treat;
means treat / tukey lines;
```

t-test

```
dm 'log;clear;output;clear';
data one;
input treat $ type$ aa $ pH $ time $ rep A B;
datalines;
          1  b          noaa       no         0min       1     10.28     10.91
          1  b          noaa       no         0min       2     10.61     10.39
          2  b          water      no         0min       1     13.41     12.46
          2  b          water      no         0min       2     10.69     10.03
          3  b          Arg        no         0min       1     11.27     14.26
          3  b          Arg        no         0min       2     14.41     12.73
          4  b          Lys        no         0min       1      15.1     14.14
          4  b          Lys        no         0min       2     11.86     13.32
          5  b          His        no         0min       1     12.01     13.01
          5  b          His        no         0min       2     11.35     11.98
          6  b          noaa        3         30min      1      9.22      9.38
          6  b          noaa        3         30min      2      9.44      9.74
          7  b          Arg         3         30min      1     11.04     11.29
          7  b          Arg         3         30min      2     10.84     10.71
          8  b          Lys         3         30min      1     11.93     11.63
          8  b          Lys         3         30min      2     11.34     12.01
          9  b          His         3         30min      1      11.1     10.79
          9  b          His         3         30min      2     10.42     10.46
         10  b          noaa       10         30min      1     10.73     10.49
         10  b          noaa       10         30min      2     10.02      7.36
         11  b          Arg        10         30min      1        11      11.4
         11  b          Arg        10         30min      2     11.11     11.68
         12  b          Lys        10         30min      1     10.19      10.2
         12  b          Lys        10         30min      2     11.86     11.18
```

13	b	His		10	30min	1	9.5	10.42
13	b	His		10	30min	2	10.51	10.92
14	b	noaa		3	1hour	1	10.62	9.48
14	b	noaa		3	1hour	2	9.65	9.32
15	b	Arg		3	1hour	1	12.54	11.19
15	b	Arg		3	1hour	2	11.34	10.69
16	b	Lys		3	1hour	1	11.16	14.41
16	b	Lys		3	1hour	2	11.83	11.8
17	b	His		3	1hour	1	13.12	11.97
17	b	His		3	1hour	2	10.6	10.92
18	b	noaa		10	1hour	1	8.28	9.1
18	b	noaa		10	1hour	2	8.79	9.37
19	b	Arg		10	1hour	1	13.11	12.87
19	b	Arg		10	1hour	2	10.74	10.03
20	b	Lys		10	1hour	1	12.99	12.62
20	b	Lys		10	1hour	2	10.09	10.56
21	b	His		10	1hour	1	12.39	13.36
21	b	His		10	1hour	2	10.8	10.7
22	e	noaa	no		0min	1	12.11	12.33
22	e	noaa	no		0min	2	12.28	12.16
23	e	water	no		0min	1	13.12	12.58
23	e	water	no		0min	2	13.59	13.67
24	e	Arg	no		0min	1	13.14	13.98
24	e	Arg	no		0min	2	16.8	12.55
25	e	Lys	no		0min	1	13.79	13.28
25	e	Lys	no		0min	2	13.96	13.89
26	e	His	no		0min	1	12.7	13
26	e	His	no		0min	2	13.37	12.55
27	e	noaa		3	30min	1	11.71	10.16
27	e	noaa		3	30min	2	11.96	12.15
28	e	Arg		3	30min	1	13.36	12.21
28	e	Arg		3	30min	2	13	13.31
29	e	Lys		3	30min	1	11.04	11.89
29	e	Lys		3	30min	2	12.67	13.09
30	e	His		3	30min	1	11.46	13.16
30	e	His		3	30min	2	12.61	13.14
31	e	noaa		10	30min	1	15.11	13.49
31	e	noaa		10	30min	2	14.08	13.91
32	e	Arg		10	30min	1	12.55	12.71
32	e	Arg		10	30min	2	13.15	12.09
33	e	Lys		10	30min	1	12.28	11.69
33	e	Lys		10	30min	2	11.65	12.13
34	e	His		10	30min	1	12.11	12.75
34	e	His		10	30min	2	12.64	12.6
35	e	noaa		3	1hour	1	12.49	13.41

```
35  e  noaa  3  1hour  2  12.46  11.78
36  e  Arg   3  1hour  1  14.14  13.42
36  e  Arg   3  1hour  2  14.14  14.53
37  e  Lys   3  1hour  1  12.03  12.38
37  e  Lys   3  1hour  2  12.6   11.9
38  e  His   3  1hour  1  13.05  12.65
38  e  His   3  1hour  2  15.36  13.91
39  e  noaa  10 1hour  1  12.62  12.99
39  e  noaa  10 1hour  2  14.14  13.13
40  e  Arg   10 1hour  1  13.62  13.34
40  e  Arg   10 1hour  2  14.25  13.06
41  e  Lys   10 1hour  1  12.86  13.6
41  e  Lys   10 1hour  2  13.61  14.5
42  e  His   10 1hour  1  13.25  13.79
42  e  His   10 1hour  2  13.28  13.49
;
proc ttest;
class type;
var A--B;
run;
```

VITA

Jonathan Michael Futch was born in Fargo, North Dakota, in 1979. He graduated from Louisiana State University in Baton Rouge, Louisiana, with a Bachelor of Science degree in food science in December 2004. He then studied food science at Louisiana State University in Baton Rouge, Louisiana, where he is currently a candidate for a master's degree. Jonathan will receive the master's degree in food science in August 2009.